JOURNAL OF AGRICULTURAL RESEARCH

VOL. 58 WASHINGTON, D. C., JUNE 1, 1939 NO. 11

ANATOMICAL STUDIES OF ROOT AND SHOOT PRIMORDIA IN 1-YEAR APPLE ROOTS[1]

By E. A. SIEGLER, *pathologist*, and J. J. BOWMAN, *assistant pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Propagation of many horticultural plants is accomplished by so-called cuttings of either stem or root pieces. The degree of success attained in producing a new plant is usually dependent upon the response of the cutting in forming and developing organs that were not present when the cutting was taken from the parent plant.

In general, tree-fruit varieties are propagated either by budding or by grafting because the rooting of stem cuttings is not commercially practicable. By means of root cuttings, however, the practicability of building up populations of clons from apple (*Malus*) seedlings has been demonstrated by Luke (*6*),[2] Yerkes (*18*), and others. Shoots that have developed from root cuttings have also been readily rooted (*10, 12, 13*).

Since the report by Shaw (*9*) on the use of the nurse-root method, many workers have grown apple varieties on their own roots and thus have obtained a source of root cuttings of named varieties. Propagation by means of root cuttings, however, has not been so successful with commercial varieties as with certain seedlings (*15, 17, 19*). A summary of the results in this country up to 1930 is available (*1*). More recently, encouraging results, at least with some varieties, have been reported by Upshall (*16*).

Attention has been called to the need for an understanding of the structural features of a plant in order to interpret the responses observed in propagation by vegetative means (*8*). The anatomical study presented here on the origin and development of root and shoot primordia in the apple root may serve as an approach to subsequent experimentation on the vegetative propagation of apple roots from known varieties.

REVIEW OF LITERATURE

The comprehensive investigations dealing with the ontogeny of apical meristems by Priestley and Swingle (*8*) mark an important advance in the manner of treating the subject of vegetative propagation. The complete review given by these workers of investigations on the general problem eliminates the need of extensive comments on investigations previous to the publication of their bulletin.

Recently there has been a definite attempt on the part of investigators to determine the earlier stages in the organization of groups

[1] Received for publication October 13, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 802.

of cells that eventually may develop into root primordia (*2, 3, 4, 11*). Most of the investigations have been made on the origin of adventive roots on stem cuttings of various plants that were allowed to callus under the usual conditions for propagation. In rose stem cuttings, Carlson (*3*) clearly demonstrated that root primordia are initiated by small groups of cells in parenchymatous tissue in the secondary phloem. In raspberry, Sudds (*14*) reported that root primordia in various types of stem cuttings and in tip-layered canes are most frequently associated with branch and leaf traces. The origin of root primordia in pith tissue has been observed by several workers (*4, 14*). More recently, Smith (*11*) has furnished a concise report of investigations on the initiation of root primordia in stem cuttings of various plants, and summarizes the findings of the various workers. If the conclusions of the workers are accepted, it appears that root primordia may originate in practically any tissue, depending upon the plant examined. Unfortunately, the evidence has not always been supported by well-defined illustrations, and frequently conclusions have been based upon observations on relatively mature primordia.

Stoutemyer (*12*) has recently reviewed the subject of regeneration in apple and has reported on the anatomical relations of root and shoot primordia. As a result of the action of various chemicals associated with the formation of root primordia, there is opened a field which has furnished new lines of attack on this problem (*5*).

The work reported here deals with the initiation and subsequent stages in development of root and shoot primordia which, according to the writers' observations, occur as preformed meristematic "cushions" on roots of 1-year apple seedlings. These primordia may continue growth under suitable conditions after the piece of root containing them has been separated from the parent plant. They are considered as adventive under the broad interpretation of the term (*8*) in that they do not arise in a normal acropetal succession on the young root.

MATERIALS AND METHODS

Most of the material used was obtained from roots of apple seedlings grown in the greenhouse. Material from this source was fixed in October and later sectioned in paraffin by the usual histological methods; butyl alcohol was frequently used in the dehydrating series. Flemming's triple stain was used. Numerous observations were also made on roots of seedlings fixed when in a dormant condition after completing the first year's growth under field conditions.

In order to determine the relative time of emergence of root and shoot primordia, root cuttings were made from clonal material of Delicious apple and Florence crab. These root cuttings, approximately 0.2 to 0.5 cm in diameter and 7 to 8 cm long, were tied in bundles of approximately 50 each and placed in a mixture of moist sand and sawdust in the greenhouse. In addition, root cuttings of a clon of a crab apple, Vermont 323, which, in common with most other seedlings is known to propagate readily under field conditions, were used for comparison.

RESULTS

The root cuttings were examined April 11, following a period of 1 month in the sand-sawdust medium.

The results given in table 1 show the variation that may be encountered in root cuttings of different clons kept under conditions favorable for growth. Past experience has shown that larger stands may be expected from root cuttings of Florence crab than of Delicious apple. The fact that 77 percent of the Florence crab cuttings were rooted, as compared with 38 percent in the Delicious apple cuttings, is significant, especially as these figures approximate the stands generally obtained in the field for these two varieties. It seems reasonable to conclude that rooting, either in advance of the pushing of shoots or synchronous with it, is necessary for the successful propagation of root cuttings. Obviously, root cuttings that push both roots and shoots at the same time are the most readily propagated. Practically every cutting of the Vermont 323 clon, held under the same conditions but in larger numbers than the other clons, developed shoots and roots.

TABLE 1.—*Condition of root cuttings after 1 month in sand-sawdust medium*

Clonal variety	Root cuttings				
	Total examined	With roots and shoots	With roots only	With shoots only	With no roots or shoots
	Number	*Percent*	*Percent*	*Percent*	*Percent*
Delicious apple	316	31	7	22	40
Florence crab	221	20	57	3	20

ANATOMY OF THE PRIMARY ROOT

The primary root of the apple is usually characterized by a tetrarch radial protostele (pl. 1). The pericycle, at the stages shown in plate 1 and plate 2, *A*, is two layers thick, although a single-layered pericycle has been reported (*12*). The cell walls of the outer layer become somewhat thickened. The appearance of these cells does not suggest meristematic activity, and, owing to the early sloughing off of the endodermis, this outer pericyclic layer functions as a protective tissue by the time secondary growth is evident.

With the beginning of secondary growth the cells of the inner layer of the pericycle (pl. 2, *B*) begin an active tangential division, and a typical phellogen is formed by this layer. At this stage the walls of the cells in the outer layer of the pericycle are greatly thickened. This layer now resembles an endodermis; the original endodermis, however, has practically disappeared. The collapse and suberization of the cell walls of this endodermlike layer of the pericycle, accompanied by cell division in the region which was formerly the inner layer of the pericycle, marks the beginning of periderm formation (pl. 2, *C*). Fibers originate in the region capping the phloem (pl. 2, *C*) and appear to become differentiated centripetally. Because of their position, method of differentiation, and the fact that there is a rather sharp line of demarcation between them and the large parenchymatous cells of the pericyclic region lying exterior to them, they are considered part of the phloem rather than the pericycle (*12*).

Secondary roots have their origin in the pericycle opposite the protoxylem points (pl. 3, *A*). By the time the differentiation of primary tissues is complete but before secondary growth is initiated in any given transverse zone, the secondary roots have become definitely organized and have made considerable growth (pl. 3, *B*).

ADVENTIVE ROOT PRIMORDIA

The presence of adventive root primordia in most seedling apple roots can be inferred from the fact that new roots appear in regions where considerable secondary growth has been made. Histological studies demonstrate the presence of these primordia in various stages of development, as shown in plates 4, 5, and 6. Although lack of morphological uniformity makes it difficult to characterize the ontogeny of a typical initial, the illustrations indicate a progressive continuity between the very early stages and the later stages where the primordia are more completely organized.

The initiating cells are consistently found in close association with the cambial region; they are frequently associated with xylem rays (pls. 4, *A*; 6, *C*). The structure shown in plate 4, *A*, represents the earliest stage observed that can be identified with a reasonable degree of certainty. The two cells with large nuclei, at *a*, are the result of a radial division of a ray cell or a ray initial in line with the cambium. The ray cells inside have remained meristematic instead of enlarging. Additional divisions in rapid succession in the two cells with large nuclei and their progeny should result in a structure similar to the primordium shown in plate 6, *A*. The fact that these cells (pl. 4, *A*) were found in a series of sections in which numerous primordia were observed and that they lie contiguous to thick-walled cells with deeply stained contents is supporting evidence that they are initiating a primordium. Similar conditions where the apical region of the primordium is bordered by thick-walled cells have been noted to occur rather consistently (pl. 4, *B*, *C*; pl. 6, *A*).

As the young primordium develops (pls. 4, *B*, *C*; 5, *B*, *C*), there is a tendency for the apical cells to have large nuclei and to divide frequently. Later stages in development (pl. 5, *B*, *C*) show some progress toward differentiation of a definite growing point, which, however, may not be localized until the stages shown in plate 6, *A*, *B*, are reached.

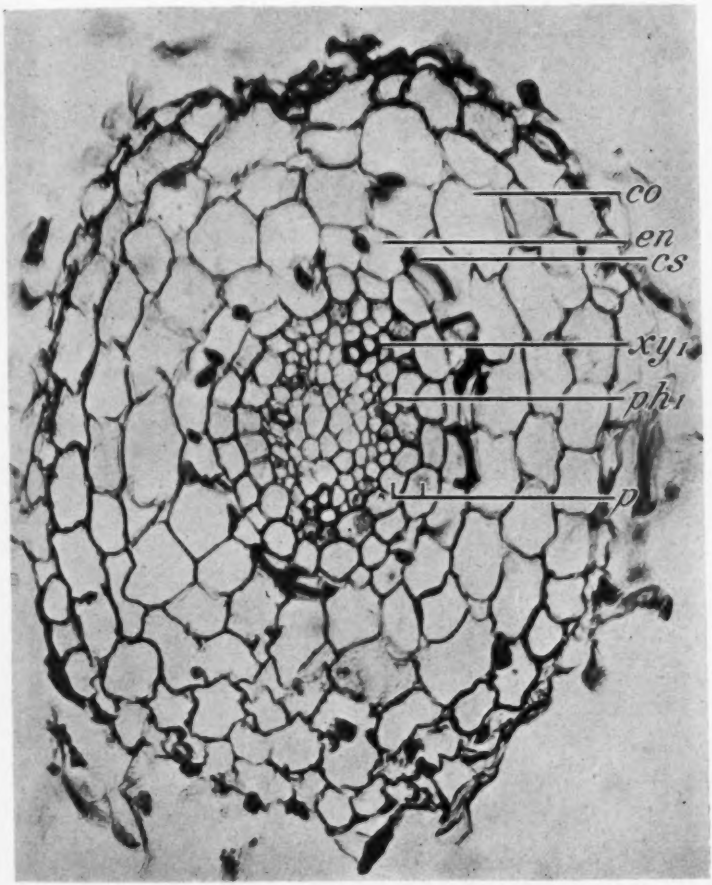
When primordia make but relatively slight forward extension, they present a "deep-seated" appearance with respect to the line of the cambium (pl. 5, *A*, *C*). There is a possibility, however, that this appearance results from partially matured ray parenchyma cells which have resumed a meristematic condition. The thin-walled meristematic condition of the cells in the xylem ray (pl. 6, *C*) is perhaps more suggestive of a resumption of meristematic properties by partially matured cells.

A comparatively few primordia were observed in which the initiating cells seemed to be several cells distant from the cambium (pl. 6, *D*), but it is difficult to determine accurately the origin of primordia that have reached this stage of development.

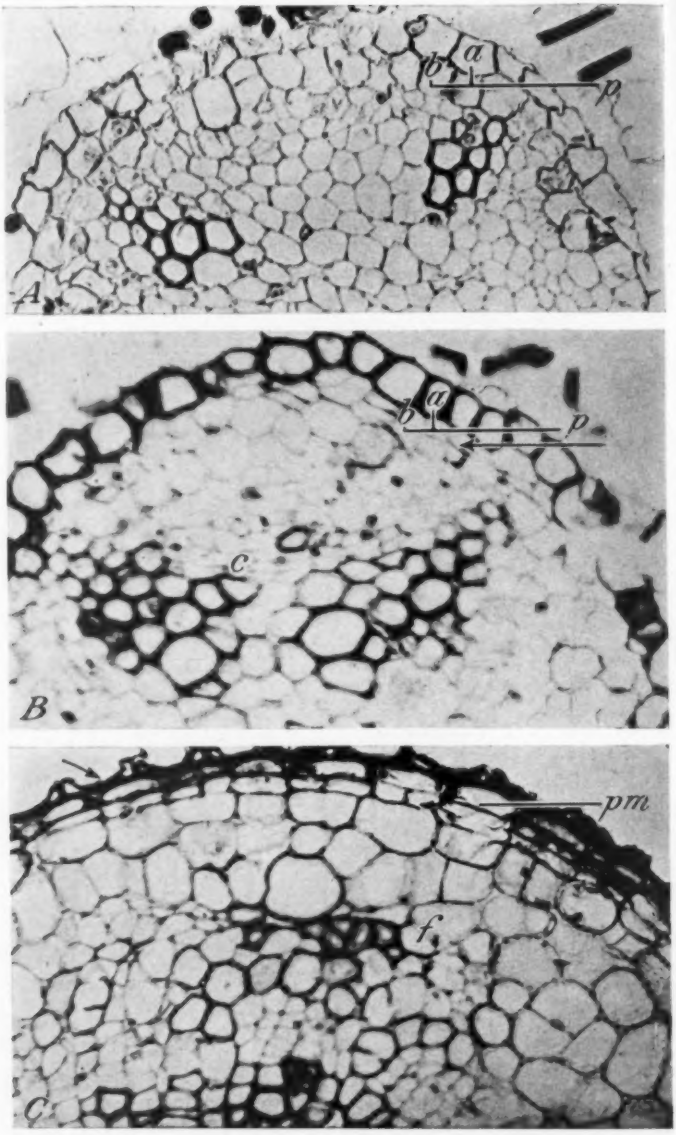
ADVENTIVE SHOOT PRIMORDIA

In contrast to the number of investigations on the origin of root primordia, there has been relatively little work on the origin of shoot primordia. Investigations to date have been summarized (*8*, *12*), but it is difficult to draw general conclusions.

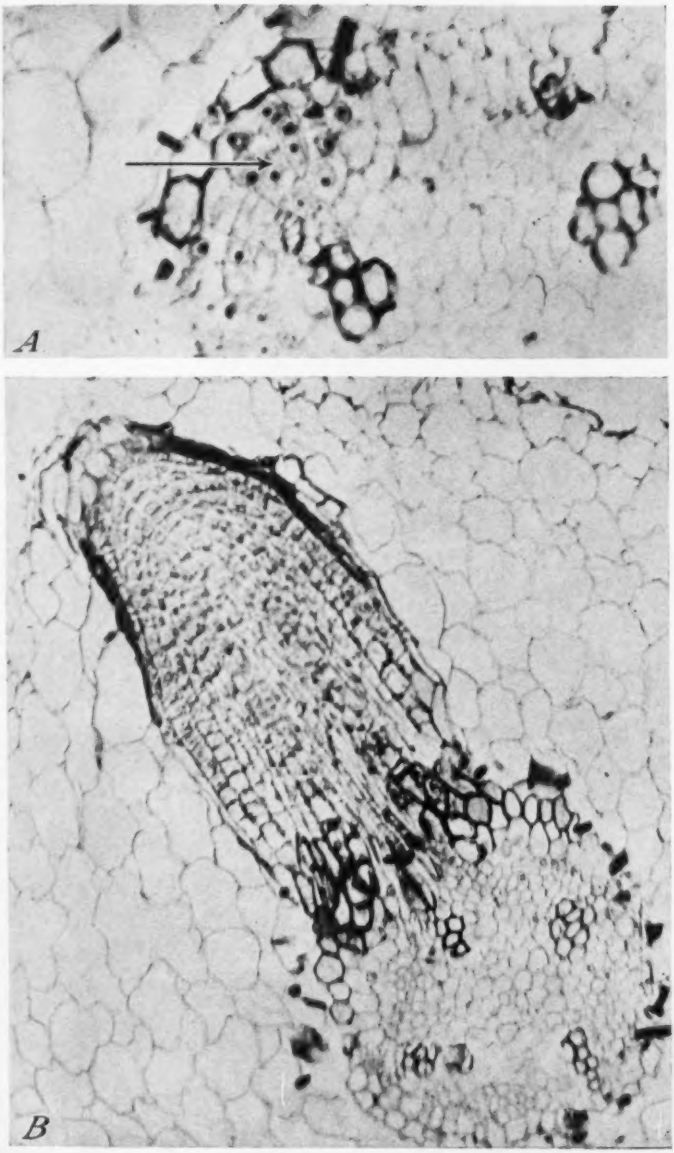
The illustrations were selected to show progressive stages in the development of shoot primordia from a small group of initiating cells to a completely organized structure (pls. 7–11). In the material used



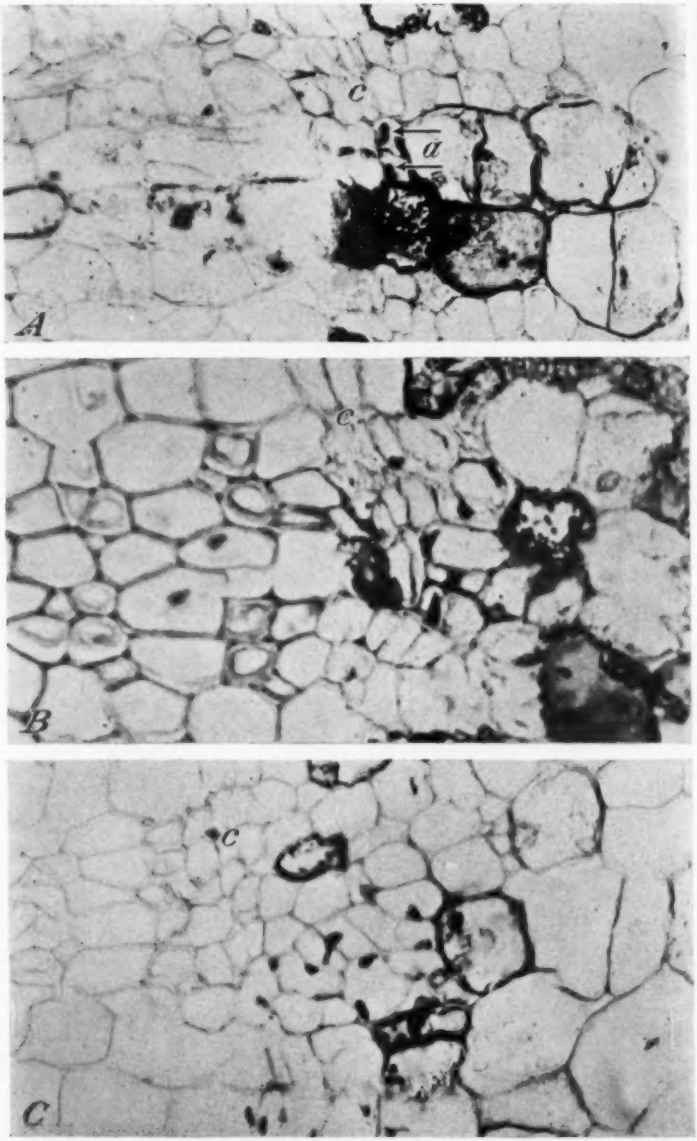
Transverse section of primary root: xy_1, Primary xylem; ph_1, primary phloem; *p*, two-layered pericycle; *en*, endodermis with the prominent Casparian strips (*cs*) on the radial and transverse walls; *co*, cortex. × 600.



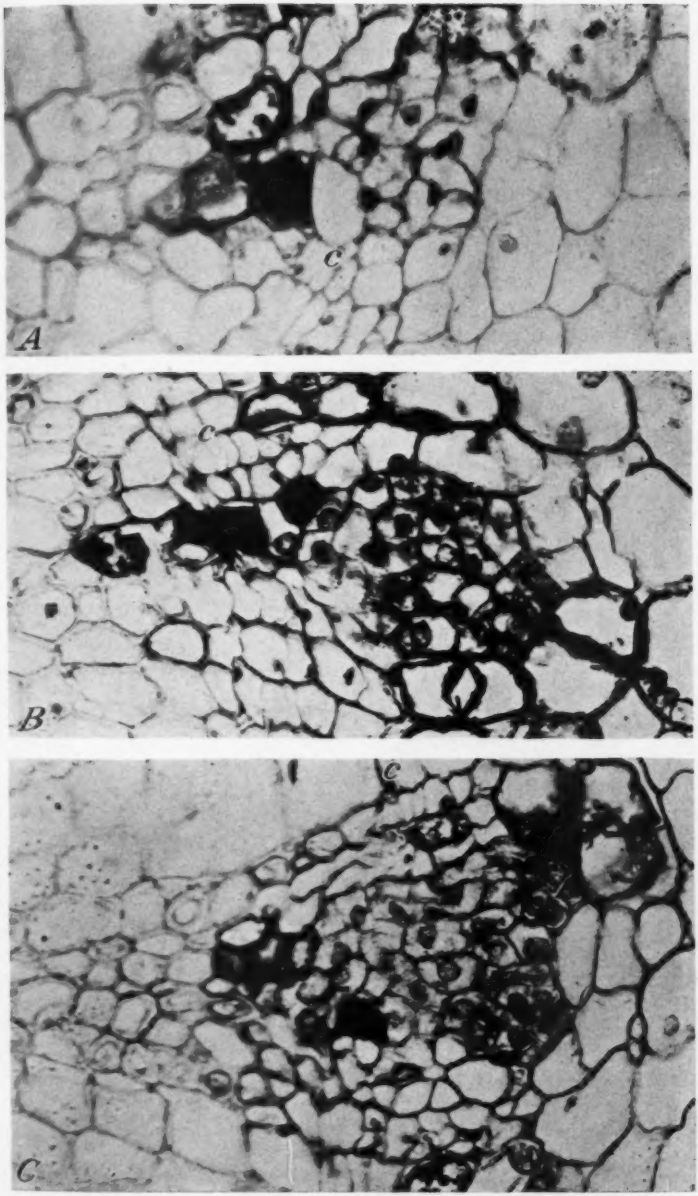
A, A late stage in primary growth. The endodermis has practically disappeared. The cells of the outer layer of the pericycle (*p*), at *a*, have comparatively thick walls; the inner layer of the pericycle (*p*) is shown at *b*. × 600. *B*, From the derivatives of the cambium (*c*) xylem and phloem elements are being differentiated. The cell walls of the outer layer of the pericycle (*p*), at *a*, are greatly thickened; this layer now resembles an endodermis. The inner layer of the pericycle (*p*), at *b*, shows numerous tangential division (indicated by arrow) and is beginning to develop a phellogen. × 600. *C*, The collapsed cells (indicated by arrow) of the original outer layer of the pericycle, together with the activity of the phellogen, mark the beginning of periderm formation (*pm*). Fibers (*f*) are present, capping the phloem elements. × 425.



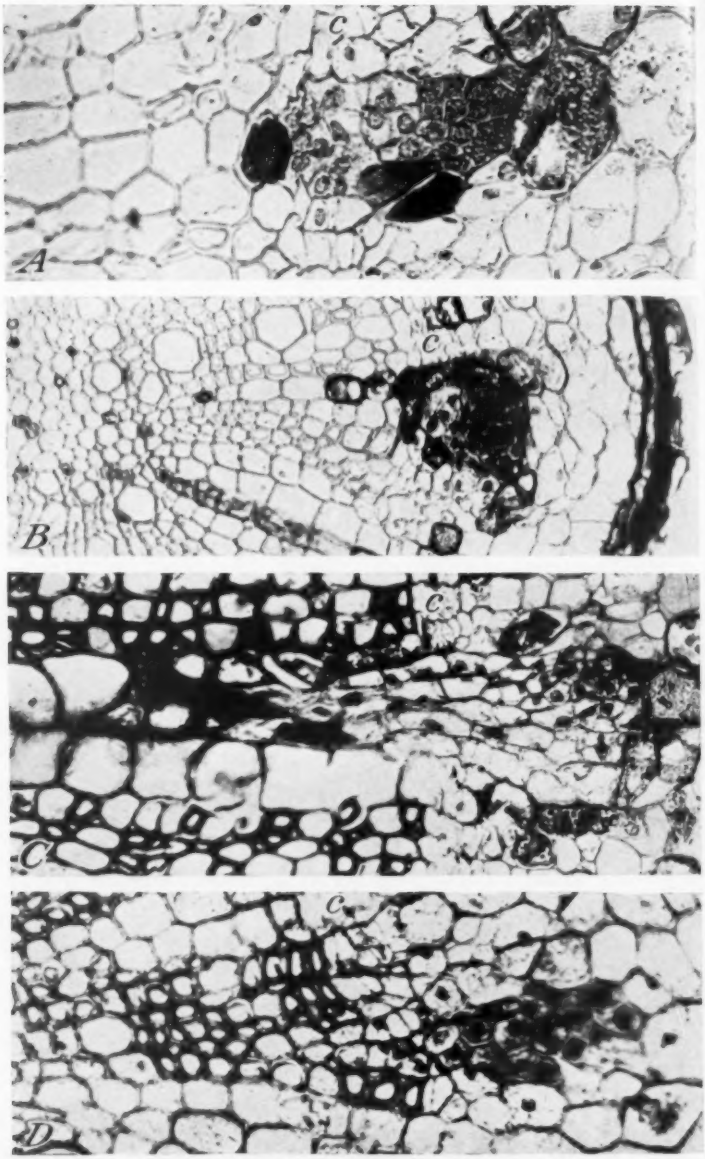
A, The initiation of a lateral root from the inner layer of the pericycle during primary growth, × 600; *B*, the lateral root is definitely organized before secondary growth in the parent axis, × 250.



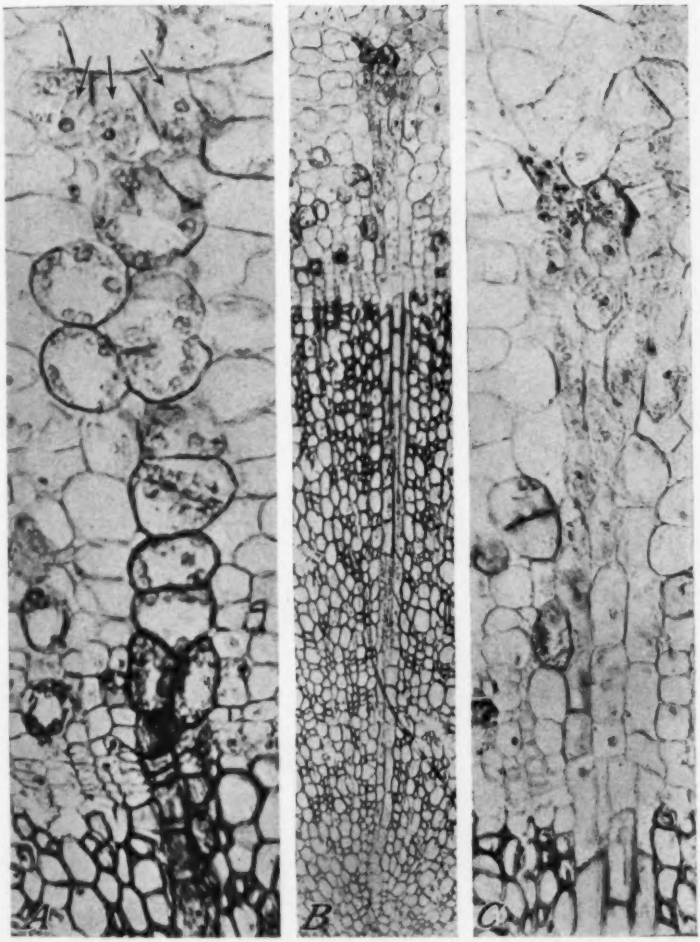
Transverse sections of apple roots showing origin of adventive root primordia in proximity to the cambial region (*c*), × 575: *A*, An early stage of a primordium, indicated by the two cells with large nuclei (indicated by arrows) at *a*, which have resulted from a radial division of a ray cell in a line with the cambium; *B*, *C*, early stages in the formation of primordia, whose apexes are partially bordered by thick-walled cells containing deeply stained contents.



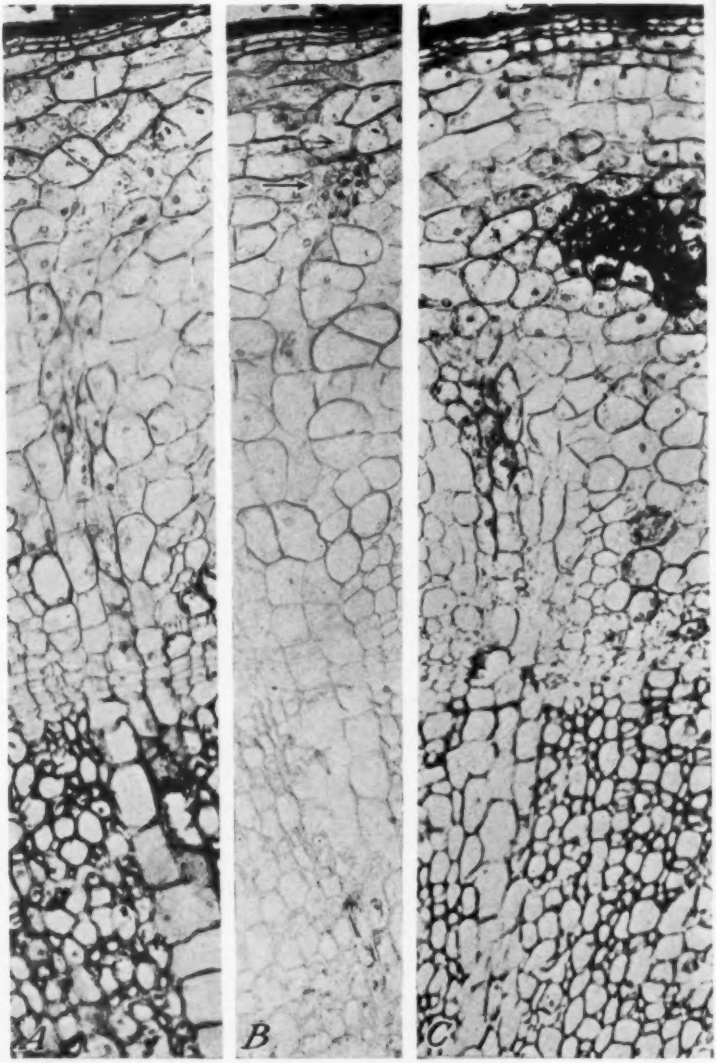
Transverse sections of roots showing later stages of primordia. *A*, × 550. *B*, *C*, The apical areas which contain cells with large nuclei are becoming organized into growing points. Present line of the cambium is at *c*. *B*, × 575; *C*, × 450.



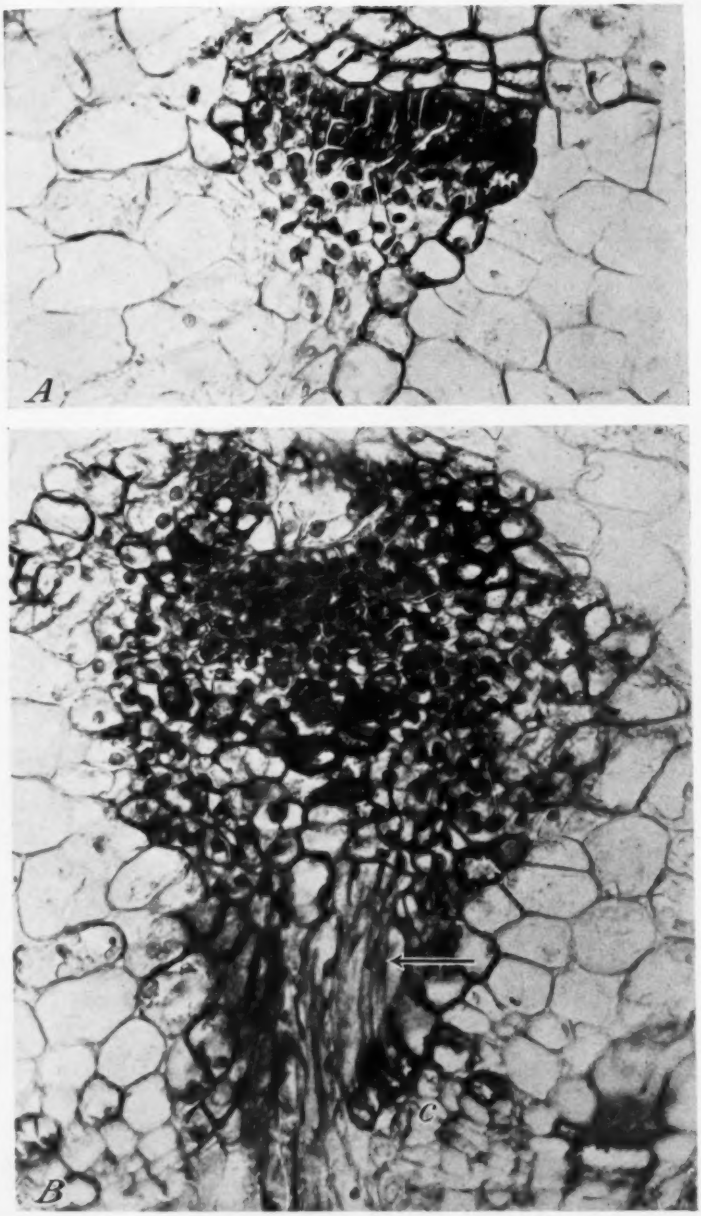
Transverse sections of roots showing adventive primordia in various stages: *A*, Organization of the growing point, × 575; *B*, a "mature" primordium, × 250; *C*, ray parenchyma cells in xylem becoming meristematic, × 325; *D*, a type of primordium, observed infrequently, in which the initiating cells may not have been intimately associated with the cambium (*c*), × 425.



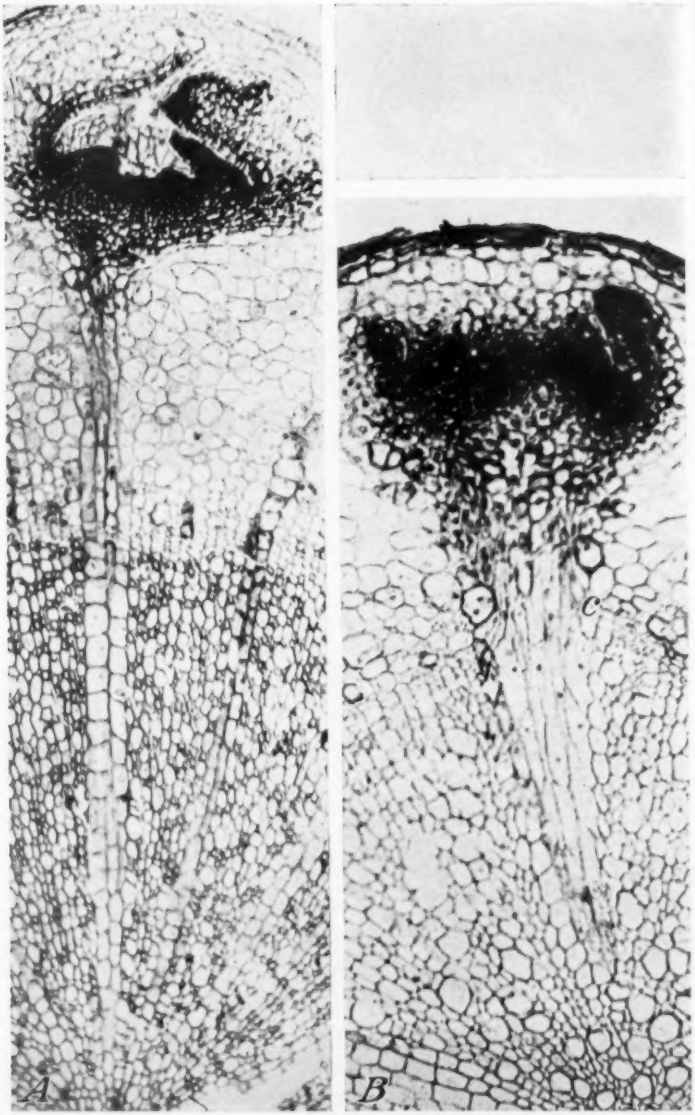
Transverse sections of roots showing early stages in the organization of adventive shoot primordia and the relation to the vascular rays. *A*, This condition probably represents a very early stage in the organization of a shoot primordium. At the distal end of a row of well-defined ray cells are three thin-walled cells (indicated by arrows) which contain small but deeply stained nuclei. × 325. *B*, A ray in which meristematic activity is especially pronounced at the apex in the phloem region. × 100. *C*, Phloem area of ray in *B* enlarged. × 250.



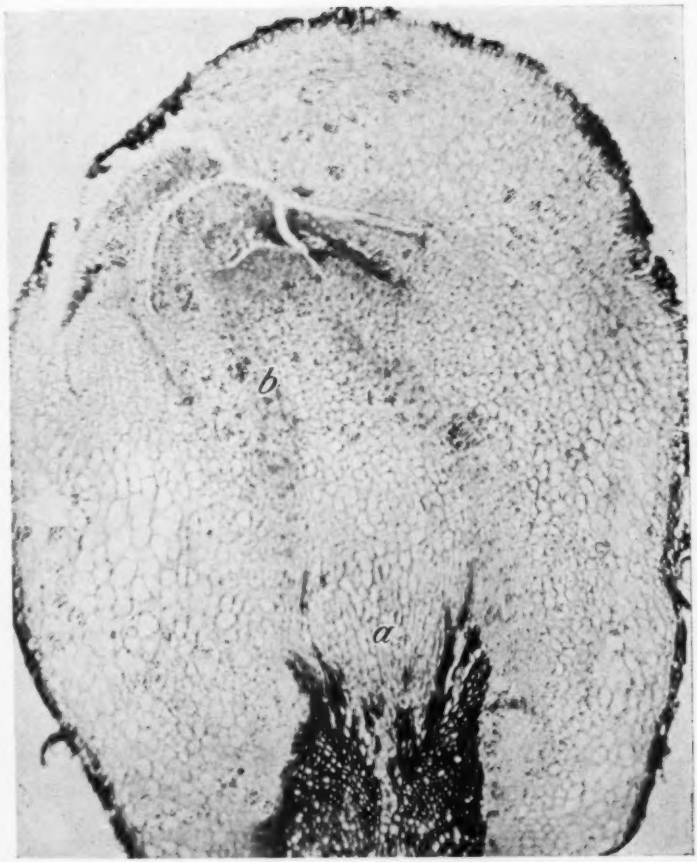
Transverse sections of root, × 175. *A*, Meristematic activity of ray cells. *B*, Serial section cut approximately 50μ distant from the section shown in *A*. The group of cells (indicated by arrow) with prominent nuclei is at the apex of the ray cells shown in *A*. *C*, A general relationship of the dense aggregation of cells at the apex of the primordium to the vascular ray is indicated.



Transverse sections of roots showing shoot primordia: *A*, A single-layered dermatogen is being differentiated, × 450; *B*, foliar initials are forming and, at the base of the primordium, the three elongated cells (indicated by arrow) will presumably function as cambium (*c*) to effect a vascular union between the primordium and the xylem of the parent tissue, × 350.



Transverse sections of roots showing shoot primordia. *A*, The distal (apical) region of the primordium is completely organized, but there is comparatively little change in the parts of the ray behind this apical region. × 100. *B*, Efficient "connections" are established between the apical region and the xylem of the parent root. Elongated meristematic cells at the proximal part of the primordium are in line with the cambium (*c*) of the parent tissue. × 150.



Transverse section of root showing an adventive shoot primordium as it is about to emerge from the parent root. The original cambial cylinder surrounding the xylem is broken, and growth to the inside has formed a large gap at *a*. The present line of the cambium enclosing this gap is continuous with the line of the procambium, which is differentiating protoxylem at the point indicated at *b*. × 75.

very few early stages were found, and it would be desirable to make additional studies on these early stages.

Shoot primordia were almost invariably found in association with vascular rays. In the apple root these rays are prominent and can be readily recognized as a continuous tissue extending from the protoxylem to a region near the periphery of the root. Meristematic activity in several ray cells near the periphery, accompanied generally by increased activity centripetally in the ray cells, appears to be the mechanism favorable for the initiation of the primordia.

In plate 7, *A*, the ray cells in the phloem are fairly conspicuous. This of course is normal, but in this case the three cells that mark the apex appear to be more meristematic than usual. The nuclei in these three cells are more deeply stained, although they are somewhat masked by the amyloid cell contents; the cell walls are very thin and in some portions are indistinguishable. This condition suggests a resumption of meristematic activity by these cells, and therefore this structure is tentatively identified as a very early stage of a shoot primordium.

The group of cells shown in plate 7, *B*, *C*, can be definitely identified as an initial stage. The nuclei at the apical region are very small but have taken an intense stain; the cell walls are barely discernible. Meristematic activity is also evident behind this region but decreases progressively toward the cambial region.

The aggregation of cells, with small, deeply stained nuclei, near the periphery in plate 8, *B*, actually marks the apex of the primordium shown in plate 8, *A*. The relationship of these areas can readily be visualized by figuratively superimposing this group of cells (pl. 8, *B*) upon a corresponding area of the ray in plate 8, *A*.

With the increase in size, it obviously becomes more difficult to secure orientation to include representative cross sections. Thus the relation of the dense, meristematic apical region of the primordium to the meristematic ray cells behind it (pl. 8, *C*) appears somewhat indefinite. Later stages in the development of primordia are shown in plates 8, *C;* 9, 10, and 11. From the unorganized mass of cells (pl. 8, *C*), the apical end of the primordium has differentiated a dermatogen (pl. 9, *A*), and foliar initials are noted in the stage shown in plate 9, *B*. The growing point has become organized in a manner similar to that described for syringa (*8*). The region of the growing point may be well organized in the absence of coordinated activity of the cells in the regions behind it (pl. 10, *A*). On the other hand, a primordium whose apical region is in a similar stage of development but which also shows complete organization as a unit with the parent tissue is shown in plate 10, *B*. Presumably this latter type of primordium, because of the completeness of its connections with the vascular tissue of the parent axis, is well adapted for survival upon emergence from the parent tissue. This anatomical consideration may explain why in shoots that emerge from root cuttings there is mortality of some, and variation in growth of others.

Ultimately, connections between the primordium and the vascular tissue of the parent root are established by differentiation of meristematic cells. Plates 9, *B*, and 10, *B*, show tagential and median longitudinal sections, respectively, of the same primordium. The elongated meristematic cells at the base of the primordium are oriented at right angles to the cambium of the parent tissue and presumably

151679—39——2

will establish a cambium cylinder at the proximal part of the primordium. By the time the stage of development shown in plate 11 is reached, a considerable amount of xylem has been laid down and the line of the cambium is continuous with the procambium from which protoxylem has developed at *b*.

It should be noted that the primordium shown in plate 11 was formed toward the end of the growing season and remained as a semidormant cushion during the dormant period of the plant. Upon resumption of suitable growing conditions in the spring, preformed primordia of this type develop rapidly. Numerous leaf primordia are formed before the shoot emerges from the cortex.

DISCUSSION AND CONCLUSIONS

In apple roots, so-called preformed root and shoot primordia are developed on the current season's growth. There is considerable variation in the frequency of occurrence of these primordia, not only in seedlings of different parentage but among different clons. The success attained in propagating root cuttings of clons is largely dependent upon the presence of these preformed primordia in the root cutting when it is taken from the parent plant. The presence of shoot primordia in particular can be frequently detected with the unaided eye by the end of the growing season. These primordia, occurring as cushions at numerous points on the roots, are generally found in abundance on seedlings of crab apples, including named varieties such as Florence, and on seedlings of commercial varieties. Observations over a number of years have led the writers to the conclusion that in general cuttings from roots that exhibit macroscopically a large number of primordia cushions are propagated most successfully.

A correlation of the anatomical studies reported here with the investigations of most other workers is difficult because of lack of similarity in the material used. Priestley and Swingle (*8*) present detailed anatomical analyses on a number of plants. Investigations in general have dealt with the production of root primordia on stem cuttings of herbaceous and woody dicotyledons. With few exceptions (*2*), the workers previous to Priestley and Swingle were concerned with noting the presence of primordia rather than with detailed anatomical studies of their origin. Unfortunately, conclusions as to the origin of primordia have frequently been made as a result of observations on mature structures. The present discussion deals primarily with initial stages of the primordia and their growth and development in root tissues only.

It should be noted that the illustrations of primordia are taken chiefly from roots that have made approximately one season's growth and represent the conditions usually found in root pieces approximately 2 to 5 mm in diameter. A few structures that could not be definitely identified were observed.

A feature not commented upon in the remarks on the illustrations is the fact that in the regions where primordia were found in greatest numbers phloem fibers were generally lacking. Additional observations are necessary before the significance of this observation can be evaluated (*7*).

The studies on the anatomy of the young root, including primary and some secondary growth and the locus of origin of the primordia, illustrate the suggestion of Priestley and Swingle (*8*), viz, that the environment in which a cell or group of cells is located is an important factor in determining the future course of development. Owing possibly to the presence of the prominent Casparian strips, the endodermal cells do not exhibit much evidence of divisions; they are sloughed off before primary growth has ceased. In turn, the walls of the outer layer of the two-layered pericycle become greatly thickened, and this layer functions as protective tissue; the inner layer of the pericycle eventually functions as a phellogen. All adventitious primordia originate after considerable secondary growth has taken place and therefore are "endopericyclic" and, in the sense of the term as used by Priestley and Swingle, would be considered endogenous.

That adventitious root primordia were formed comparatively late in the growing season is indicated by the relative stages of organization of the more mature root and shoot primordia. Shoot primordia were usually more completely developed than root primordia. This fact may explain why many root cuttings, when planted, produce numerous shoots that may make considerable growth before the adventive roots have developed proportionately. As a result of this paucity of roots, a high mortality may result (*17*, *19*). However, with the root cuttings held in the greenhouse under conditions favorable for growth, the fact that cuttings of the Florence crab variety in general exhibited root growth in advance of shoots shows that variations may be expected not only between different clons but also between individuals of the same clon.

The evidence obtained from a study of very early stages of root primordia indicates a close association between the initiating cells and the cells of the vascular cambium, or at least their close derivatives. There is no consistent relation of the primordia to the vascular rays. The small group of cells at the apex of the young primordium almost invariably is contiguous to, and apparently delimited by, groups of parenchymatous cells possessing comparatively thick walls and deep-staining cell contents. Possibly there is here a relationship similar to that postulated for the function of the endodermis (*8*).

Because of the close association of the early stages of the root primordium with the cambium, continuity with the mature vascular tissues of the parent axis is readily established. Those root primordia that originate in a ray probably have an advantage in subsequent growth, because the parenchymatous ray cells have the potential meristematic properties which would result in differentiation of cells capable of effecting an optimum anatomical union with the xylem tissues of the parent axis.

The ontogeny of adventive shoot primordia has been discussed previously in some detail in connection with the individual illustrations. The literature dealing with the origin of adventive shoot primordia is decidedly meager and is concerned chiefly with herbaceous plants (*8*, *12*). In this paper emphasis is placed on the probable role of the vascular rays in the formation of shoot primordia. The genetic connection and homogeneity of the tissue extending from near the protoxylem points to a region near the periphery of the mother tissue apparently provide an environment favorable for initiation of

shoot primordia. Although the ray cells may differ in appearance because of their cellular environment, they are a relatively homogeneous unit in function and in structure. It is therefore not surprising that these ray cells act as a unit in the formation of the primordium. It should be noted, however, that Stoutemyer (*12*) reports that bud primordia do not arise at any definite points with respect to the vascular rays.

Manifestations of differentiation in the ray cells first become apparent in the periphery of the parent tissue, as has been discussed. The relative activity may vary in the regions destined to be the proximal and distal ends of the primordium. As a result, the vascular connections established with the parent tissue may vary greatly between different primordia. It is permissible to postulate that the establishment of adequate vascular connections presages successful growth of the young shoot.

SUMMARY

The origin and development of adventive root and shoot primordia in 1-year apple roots have been studied in order to obtain an explanation, based on anatomy, of the vegetative response.

The anatomy of the primary root is described to show the origin of normal lateral roots and the differentiation of secondary tissues.

Adventive root and shoot primordia have their origin in tissues that have made considerable secondary growth.

Adventive root primordia originate in the region of the vascular cambium, either in ray parenchyma cells in a line with the cambium or in near derivatives of cambial cells not associated with rays.

Shoot primordia are organized as a result of meristematic activity of ray parenchyma cells. Activity in a small group of ray cells near the periphery is accompanied by divisions in the posterior regions of the ray frequently extending into the xylem. The conception that the ray cells exist as a homogeneous genetic unit through the xylem and phloem regions is advanced to explain the manner in which the shoot primordium is organized.

In the material observed, shoot primordia were generally more completely organized than root primordia. This condition probably accounts for the relatively quick emergence of shoots in plantings of certain clons and the resulting mortality due to lack of roots sufficient to support the top growth. Variations in the manner in which the vascular connections are established between the shoot primordium and the parent tissue probably explain variations in the vegetative response of shoots growing from individual root cuttings.

The vegetative response of root cuttings of several clons indicates that successful propagation is largely dependent upon the ability of the cutting to push roots either in advance of shoots or concurrently with them.

LITERATURE CITED

(1) Auchter, E. C.
1930. American experiments in propagating deciduous fruit trees by stem and root cuttings. 9th Internatl. Hort. Cong. Rept. and Proc., pp. 287–296.

(2) Carlson, Margery C.
1929. Origin of adventitious roots in coleus cuttings. Contrib. Boyce Thompson Inst. 2: 39–46, illus.

(3) CARLSON, MARGERY C.
1933. COMPARATIVE ANATOMICAL STUDIES OF DOROTHY PERKINS AND AMERICAN PILLAR ROSES. I. ANATOMY OF CANES. II. ORIGIN AND DEVELOPMENT OF ADVENTITIOUS ROOTS IN CUTTINGS. Contrib. Boyce Thompson Inst. 5: 313–330, illus.
(4) CONNARD, MARY H., and ZIMMERMAN, P. W.
1931. THE ORIGIN OF ADVENTITIOUS ROOTS IN CUTTINGS OF PORTULACA OLERACEA L. Contrib. Boyce Thompson Inst. 3: 337–346, illus.
(5) KRAUS, E. J., BROWN, N. A., and HAMNER, K. C.
1936. HISTOLOGICAL REACTIONS OF BEAN PLANTS TO INDOLEACETIC ACID. Bot. Gaz. 98: 370–420, illus.
(6) LUKE, FRED K.
1898. FRUIT TREES FROM CUTTINGS. Jour. Columbus Hort. Soc. 13: 95–99.
(7) NIGHTINGALE, G. T.
1935. EFFECTS OF TEMPERATURE ON GROWTH, ANATOMY, AND METABOLISM OF APPLE AND PEACH ROOTS. Bot. Gaz. 96: 581–639, illus.
(8) PRIESTLEY, J. H., and SWINGLE, CHARLES F.
1929. VEGETATIVE PROPAGATION FROM THE STANDPOINT OF PLANT ANATOMY. U. S. Dept. Agr. Tech. Bull. 151, 99 pp., illus.
(9) SHAW, J. K.
1919. THE PROPAGATION OF APPLE TREES ON THEIR OWN ROOTS. Mass. Agr. Expt. Sta. Bull. 190, pp. [73]–96, illus.
(10) SIEGLER, E. A.
1930. EFFECT OF THE APPLE STRAIN OF THE CROWN-GALL ORGANISM ON ROOT PRODUCTION. Jour. Agr. Research 40: 747–753, illus.
(11) SMITH, A. I.
1936. ADVENTITIOUS ROOTS IN STEM CUTTINGS OF BEGONIA MACULATA AND B. SEMPERFLORENS. Amer. Jour. Bot. 23: 511–515, illus.
(12) STOUTEMYER, V. T.
1937. REGENERATION IN VARIOUS TYPES OF APPLE WOOD. Iowa Agr. Expt. Sta. Research Bull. 220, pp. [307]–352, illus.
(13) ——— MANEY, T. J., and PICKETT, B. S.
1936. SOME OBSERVATIONS ON THE PRODUCTION OF OWN-ROOTED APPLE STOCKS FROM ROOT CUTTINGS. Amer. Soc. Hort. Sci. Proc. (1935) 32 (v. 33): 350–354, illus.
(14) SUDDS, R. H.
1936. THE ORIGIN OF ROOTS IN SEVERAL TYPES OF RED AND BLACK RASPBERRY STEM CUTTINGS. Amer. Soc. Hort. Sci. Proc. (1935) 32 (v. 33): 380–385, illus.
(15) UPSHALL, W. H.
1931. THE PROPAGATION OF APPLES BY MEANS OF ROOT CUTTINGS. Sci. Agr. 12: 1–30, illus.
(16) ———
1936. PROPAGATION RESPONSE FROM ROOT CUTTINGS PLANTED WITH THE PROXIMAL END PROJECTING ABOVE THE MEDIUM. Sci. Agr. 17: 146–147, illus.
(17) ——— and GARDNER, F. E.
1929. RESPONSES OF VARIETY AND SEEDLING ROOTS TO ATTEMPTS AT PROPAGATION. Amer. Soc. Hort. Sci. Proc. (1929) 25: 172–174.
(18) YERKES, G. E.
[1924]. EXPERIMENTS IN THE PROPAGATION OF FRUIT-TREE STOCKS. Amer. Soc. Hort. Sci. Proc. (1923) 20: 241–244.
(19) ———
[1927]. PROPAGATION OF APPLE BY ROOT CUTTINGS AND LAYERS. Amer. Soc. Hort. Sci. Proc. (1926) 23: 93–98.

BROWN NECROSIS, A DISCOLORATION ASSOCIATED WITH RUST INFECTION IN CERTAIN RUST-RESISTANT WHEATS [1]

By E. S. McFADDEN [2]

Associate agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In recent years many conflicting statements concerning the nature and symptoms of the black chaff disease of wheat (*Triticum aestivum* L., syn. *T. vulgare* Vill.), usually attributed to the organism *Bacterium translucens* var. *undulosum* Smith, Jones, and Reddy, have appeared in the literature on cereal diseases. Several investigators have reported that the rust-resistant varieties Hope and H–44 and many of their derivatives are very susceptible to attack by this organism. Because of the prevalence of a so-called black chaff in the rust-resistant hybrid segregates from these wheats, some concern has been expressed over the possible results should such wheats become widely grown. For this reason many strains of wheat descended from Hope and H–44 that appear to be peculiarly susceptible to black chaff are discarded every year from various wheat-breeding nurseries. The importance of knowing the true cause and probable future economic importance of the disease is, therefore, quite obvious.

This paper presents experimental data, together with certain evidence from publications of other investigators, which would seem to demonstrate that much of the so-called black chaff reported in Hope and H–44 and their rust-resistant derivatives is merely a manifestation of resistance to stem rust. In this paper, the term "brown necrosis" is applied to the melanistic reaction or discoloration that develops in certain varieties in the presence of stem rust.

REVIEW OF LITERATURE ON MELANISM IN RUST-RESISTANT WHEATS

In previous publications [3] (*10*) [4] the writer drew attention to the "association," in selections from a Yaroslav emmer × Marquis cross, of susceptibility to "black chaff" and "anthracnose" with a type of resistance to stem rust which was derived from the Yaroslav emmer parent. It was pointed out [5] that the association of resistance to

[1] Received for publication August 24, 1938. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Texas Agricultural Experiment Station. Contribution No. 490, Technical Series, Texas Agricultural Experiment Station.

[2] The writer gratefully acknowledges his indebtedness to the following institutions and individuals: The South Dakota Agricultural Experiment Station, for cooperative assistance during the first 11 years of the investigations presented herein; the Minnesota Agricultural Experiment Station, for valuable cooperative assistance in 1935; the Texas Agricultural Experiment Station, for cooperation during 1936 and 1937 which made the final studies possible; Dr. E. C. Stakman and his coworkers at the University of Minnesota, for identification of the rust forms used in the experiments; Dr. P. C. Mangelsdorf and Dr. W. N. Ezekiel, of the Texas Agricultural Experiment Station, for valuable suggestions and criticism in the preparation of the manuscript.

[3] McFADDEN, E. S. POSSIBILITIES AND DIFFICULTIES IN THE FIELD OF RADICAL WHEAT CROSSING. 1st Ann. Hard Spring Wheat Conf. N. Dak. Agr. Col., March 27, 1928, Rept., pp. 58–61. [Mimeographed.]

[4] Italic numbers in parentheses refer to Literature Cited, p. 819.

[5] McFADDEN, E. S. See footnote 3.

stem rust with susceptibility to black chaff was not complete. The belief also was expressed (*10*) that some of the pigmented areas in the vicinity of the nodes of the emmer derivatives were the result of infection by stem rust. Hart and Zaleski (*7*) have since presented evidence which supports this opinion and have also stated that some of the pigmented areas surrounding the points of infection by stem rust resembled the lesions produced by *Bacterium translucens* var. *undulosum*.

Waldron (*12*) reported an "antagonistic relationship" between black chaff and stem rust in hybrids of Hope wheat. Hart and Zaleski (*7*), however, found no antagonistic relationship between stem rust and the true black chaff disease caused by *Bacterium translucens* var. *undulosum*.

Ausemus (*1*) and Hayes et al. (*8*) reported what they considered to be a "genetic linkage" between susceptibility to black chaff and the mature-plant type of resistance to stem rust derived from Hope and H–44, but believed that their data indicated that the linkage could be broken.

Bamberg (*2*) stated that Hope and H–44 are among the varieties most susceptible to black chaff and that there is danger of the disease becoming epidemic should selections obtained from crosses with these varieties become widely grown. This opinion is typical of many that are expressed in the literature.

Greaney (*3*, *4*) found that, in a rust-resistant descendant of H–44, applications of sulphur dust prevented the development and spread of a "bacterial disease of wheat called black chaff."

More recently Hagborg (*5*) has reported that black chaff is a composite disease. He reached the conclusion that one form of the disease, which he designated as "internodal melanism," is the result of a physiologic disorder caused by exposure of the plants to unfavorable conditions. This conclusion was reached largely because the disorder had been produced in certain rust-resistant varieties by subjecting the plants to high temperatures in a humidity chamber.

In a preliminary article (*11*) the writer concluded that at least a part of the so-called black chaff disease of Hope and H–44 was the result of infection by stem rust. The present paper contains the detailed experiments and observations upon which those conclusions were based.

DESCRIPTION OF BROWN NECROSIS

Brown necrosis first appears in the form of brown or purplish-brown blotches of various sizes on the culms, peduncles, glumes, and rachises, and occasionally on the awns and leaf sheaths. On the stems, just below the nodes, the lower margins of the blotches usually are more or less distinctly striated. On the sheaths, the blotches are of less common occurrence and, when they do occur, are often in the form of halos surrounding chlorotic areas. The blotches usually appear during the second week after heading and shortly thereafter change to dark brown or nearly black accompanied by necrosis of many of the plant cells. The discolored areas usually approach their maximum development in a given variety of wheat within a few days after their first appearance, following which there is little further development within that variety except on the later maturing culms. Upon first

appearance, brown necrosis can usually be identified by its purplish-brown color, whereas lesions of those diseases with which brown necrosis is most easily confused start as water-soaked areas. The appearance of reddish-purple spots around rust pustules should not, however, be confused with brown necrosis. Furthermore, such spots are not an indication of rust resistance, as they often appear in numerous susceptible varieties of wheat that are characterized by purple pigment even in healthy culms.

EARLY OBSERVATIONS AND EXPERIMENTS WITH BROWN NECROSIS

The condition designated in this paper as brown necrosis was first observed by the writer in 1915 on the stems of the rust-resistant emmer varieties Vernal and Yaroslav. In years following, it was observed also on rust-resistant selections from crosses of these varieties and also on Acme durum and rust-resistant selections from crosses with it. The condition was at first confused with anthracnose (*Colletotrichum graminicolum* (Ces.) Wils.), a disease referred to by Wilson (*13*) the preceding year. Later observations, however, showed that it was not identical with that disease. Following these observations, numerous attempts to isolate a causal organism and to transfer the disease from stem to stem by the puncture method met with failure. Also, attempts to control the disease by various seed treatments were fruitless. It soon became apparent that the condition was closely associated with resistance to stem rust. In the belief that the association might be the result of genetic linkage, numerous attempts were made to break the linkage by backcrossing to resistant varieties. All of these efforts gave negative results.

Further observations showed that brown necrosis did not spread from infection centers within a susceptible variety. Large fields of Hope wheat often have remained practically free from the necrotic condition, which developed profusely on the same variety grown in nursery rows adjacent to rust-susceptible varieties. Also, in common with stem rust, brown necrosis usually has developed more profusely on the north sides of the culms than on the other sides. (In the Texas Gulf coast in 1936 and 1937, however, the heaviest stem rust infection and brown necrosis developed on the south sides of the culms.) All of these observations give support to the belief that the brown pigmentation may follow an arrested infection of stem rust, the inoculum of which comes from rust-susceptible varieties. Nevertheless, numerous attempts to produce the pigmentation in the plants after they were fully headed, by spraying with suspensions of rust spores in water, gave only negative results.

EXPERIMENTAL PRODUCTION OF BROWN NECROSIS

In the summer of 1932, at Redfield, S. Dak., several plants of Hope wheat growing in the field were inoculated hypodermically at different stages of growth with suspensions of stem rust spores in distilled water, in an effort to determine at what stage of development the mature-plant type of rust resistance becomes effective. It was found that when the culms were inoculated while the plants were in the early boot stage a light infection of rust developed. When inoculation was

delayed until just before the head emerged from the boot, little or no rust developed but a profuse development of brown necrosis resulted. It was at first thought that this might be due to contamination of the rust inoculum with some other organism that was responsible for the brown necrosis. It was noted, however, that rust and brown necrosis did not develop simultaneously on the same culms. Further experiments were delayed because of crop failures in 1933 and 1934.

The experiment was repeated in 1935 on the agronomy plots at University Farm, St. Paul, Minn. Here again, a light infection of stem rust developed on the Hope plants when inoculated prior to the late boot stage, but when inoculated just before the spikes emerged from the boots, brown necrosis developed instead of rust. However, since brown necrosis also developed in certain other material in all parts of the nursery at about the same time, it could not be determined definitely whether its occurrence on the inoculated plants had resulted from the inoculations or from natural infection that spread from surrounding material.

The study was resumed at the Texas Agricultural Experiment Station, College Station, Tex., in the spring of 1936. Twelve culms each of Hope and Kota × Webster (H–151) growing in the wheat nursery were inoculated hypodermically with a suspension of urediospores of stem rust of wheat collected from three widely separated infection centers on the experiment station plots. This rust was later identified by pathologists at the University of Minnesota as races 11 and 17. Hope wheat, in the seedling stage, is susceptible to both of these forms, while H–151 is moderately resistant in both seedling and mature-plant stages.

The inoculations were made by injecting the inoculum into the boots just before the spikes emerged. At the same time, the leaf sheath of the second leaf from the top was gently drawn away from the stem and a small quantity of inoculum was inserted between it and the stem. Twelve other culms each of Hope and H–151 were treated in like manner with boiled distilled water. A third set of culms in the same stage of development was left untreated. Thirteen days later the 12 culms of H–151 that had been inoculated with stem rust spores were covered heavily with small pustules of stem rust on the stems, necks, sheaths, and glumes, while the untreated culms and those that had received injections of boiled distilled water were entirely free from both stem rust and brown necrosis.

The inoculated culms of Hope were free from rust pustules on all parts exposed to sunlight but had a profuse development of brown necrosis on the glumes and necks and on the stems just below the nodes where the rust inoculum had been inserted under the leaf sheaths. On a few of the inoculated culms of Hope, fairly normal rust pustules, but no brown necrosis, developed beneath the sheaths where the tissues were not exposed to direct sunlight. The untreated culms of Hope, as well as those receiving injections of boiled distilled water, remained free from both rust and brown necrosis. Typical specimens of treated plants are shown in figures 1, 2, and 3. It is noteworthy that no other brown necrosis could be found anywhere else in the nursery at that time except in one row of Hope wheat that was located only 2 feet from the first-observed infection center of stem rust in the nursery. In this row of Hope, every culm in which the stem was

FIGURE 1.—Effect of hypodermic inoculation of Kota × Webster (H–151) in the late boot stage with urediospores of stem rust. *A* and *B*, Stems and peduncles photographed 15 days after inoculation. Note profuse development of stem rust, but no brown necrosis. *C* and *D*, Stems and peduncles injected with boiled distilled water.

exposed below the upper node showed some brown necrosis and many of the necks and glumes also were affected but no stem rust pustules could be found. An adjoining row of H–151 was heavily infected

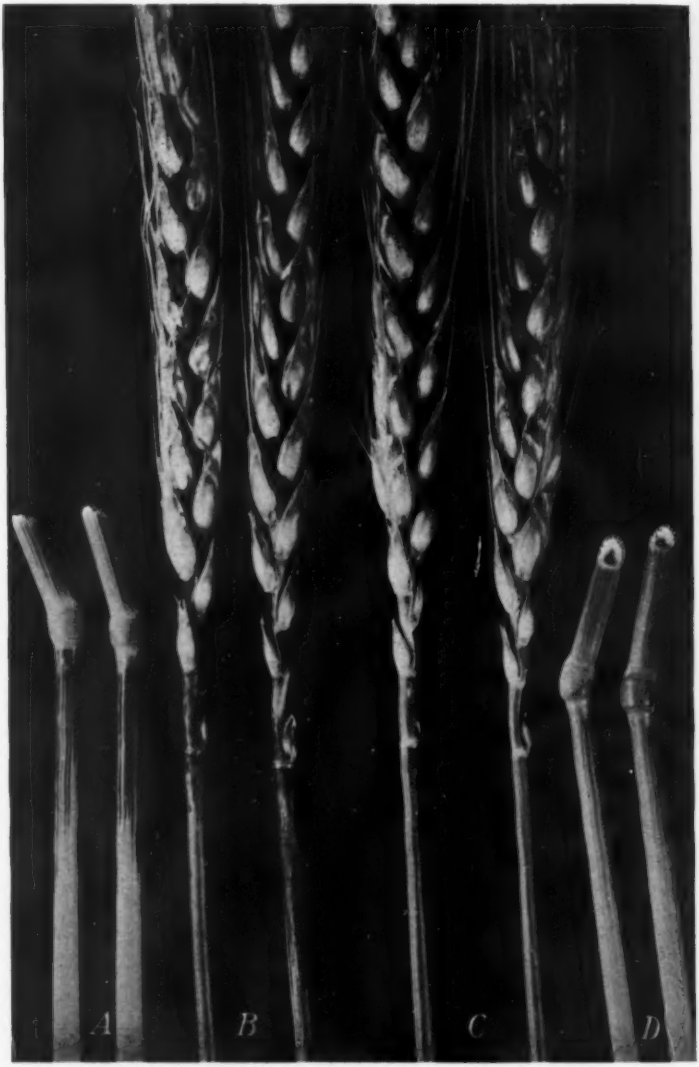


FIGURE 2.—Effect of inoculating Hope wheat in the late boot stage with urediospores of stem rust. *A* and *B*, Stems and peduncles of Hope wheat photographed 15 days after inoculating. Note the profuse development of brown necrosis, but no rust pustules. *C* and *D*, Stems and peduncles of Hope wheat checks injected with boiled distilled water.

with stem rust from the ground clear up to the tips of the awns but developed no brown necrosis. In another part of the nursery, about 100 feet south of the above-mentioned rust-infection center, no brown

necrosis whatever could be found on a row of Hope wheat, and likewise there was no stem rust on an adjoining row of H–151. Thus there appeared to be a direct relationship between the presence of stem rust on the moderately rust-resistant variety H–151 and the presence of brown necrosis on adjoining rows of Hope wheat. Figure 4 shows typical stems of Hope and H–151 from the above-described locations.

FIGURE 3.—Typical stems of Hope wheat inoculated with stem rust at the late boot stage by injecting inoculum underneath the leaf sheaths; the sheaths have been stripped off to show the results of the inoculation. Brown necrosis developed profusely between the nodes and the tops of the leaf sheaths where the stems were exposed to direct sunlight. Considerable stem rust, but only a trace of brown necrosis, developed underneath the sheaths where the tissues were not exposed to direct sunlight.

The material was photographed about 10 days after the plants headed, which was approximately 10 days before rust infection became uniformly distributed in the nursery. It is recognized that this experiment does not prove that organisms other than *Puccinia graminis tritici* Eriks. and Henn. or other conditions might not cause brown necrosis, although the relationship to rust is strongly suggestive.

OBSERVATIONS IN NURSERY PLOTS

An excellent opportunity to observe the relationship between stem rust infection and brown necrosis was also afforded in the replicated wheat nursery at College Station, Tex., in 1936. This nursery con-

sisted of four replications of 18 rust-resistant selections from a Hope × Mediterranean (T. S. 3015–63) cross, with Denton, which is rust susceptible, and a bulk population of Hope × Mediterranean (T. S. 3015–63) used as checks. The wheats were grown in triple-row groups with rows running northeast and southwest and replicated four times. The planting order was in the form of a modified Latin square. Although the planting order was partly randomized, Denton and the bulk Hope × Mediterranean fell side by side in all four replications. Since the Denton variety and many of the Hope × Mediterranean plants were susceptible to stem rust, there occurred in each replication

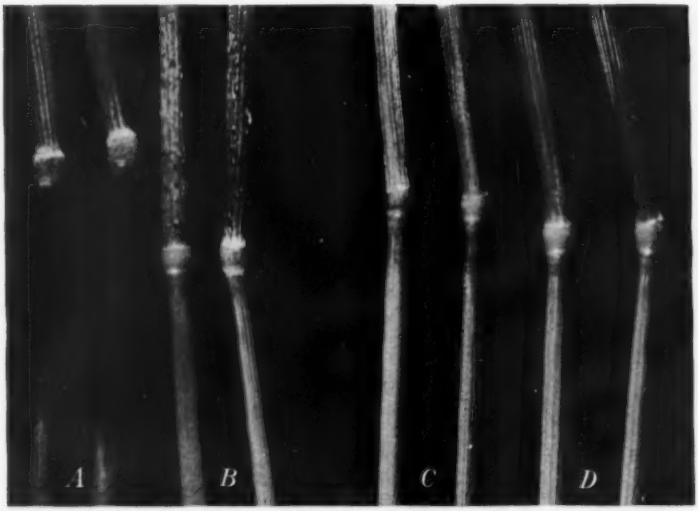


FIGURE 4.—Effect of natural rust infection on Hope and Kota × Webster (H–151): *A*, Stems of Hope wheat grown within 1 foot of an early infection center of stem rust; *B*, stems of Kota × Webster (H–151) grown in a row adjacent to *A*; *C*, stems of Hope wheat grown 100 feet south of the same infection center; *D*, stems of Kota × Webster grown in a row adjacent to *C*. Photographed about 10 days after heading.

a block of six rows of wheat in which susceptible plants occurred that served as a center from which rust spores were being disseminated after the susceptible plants had become infected. The spread of stem rust from infection centers under the conditions of southern Texas is almost entirely toward the north or northwest. It was decided, therefore, to compare the amount of brown necrosis in a given wheat selection in replications where it was grown on the northwest side adjacent to the rust-susceptible block with the amount in the same selection in replications where it was grown at various distances from susceptible blocks. The comparisons (table 1) are based on the percentage of infected peduncles in 100 culms in the center row of a three-row group. It will be observed that in every selection the percentage of culms on which brown necrosis developed was highest in the replication grown adjacent to the rust-susceptible block.

TABLE 1.—*Effect of distance from source of rust inoculum on development of brown necrosis*

Selection No.	Culms that developed brown necrosis in indicated row						
	Next to rusted block	In replications				Average	
		1	2	3	4	Actual	Comparative [1]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
41-22-1	73	----------	19	13	28	20.0	27.4
41-8-3	57	17	----------	21	37	25.0	43.9
41-57-3	33	21	[2] 29	----------	15	21.7	65.8
41-27-3 [3]	41	33	[4] 36	27	----------	32.0	78.0

[1] In terms of percentage of plot next to rusted block.
[2] Next to segregating guard row.
[3] Segregating for rust reaction.
[4] 5 feet north of segregating guard row.

OBSERVATIONS IN FIELD PLOTS

Another opportunity to observe the relation of rust inoculum to the development of brown necrosis was afforded by conditions existing in the field plots of wheat at College Station, Tex., in 1936. These plots consisted of 11 rust-resistant selections from the Hope × Mediterranean cross (T. S. 3015–63) grown in comparison with an unselected population of this cross. The plots were separated by alleys 2 feet wide, with a row of Mediterranean (T. S. 3015–105), which is very susceptible to stem rust, planted in the center of each alley. Several infection centers of stem rust had appeared in the Mediterranean wheat in the alleys by the time the Hope × Mediterranean selections started heading. These centers were marked as soon as they were found. Shortly before the wheats ripened, the development of brown necrosis on the Hope × Mediterranean selections was found to be much heavier adjacent to the rust-infection centers than elsewhere in the plots. The contrast was so distinct that one could stand at a considerable distance and locate the infection centers in the guard rows by the dark spots in the adjoining plots.

STAGE OF DEVELOPMENT OF THE HOST AT THE TIME OF RUST INOCULATION AND DEVELOPMENT OF BROWN NECROSIS

Heavy infections of stem rust often occur on susceptible varieties in the spring wheat area with but little brown necrosis developing on most of the rust-resistant types derived from Hope and H–44 in the same nursery. Under such conditions, it has been observed by the writer in several years that the later maturing rust-resistant strains developed more brown necrosis than the earlier maturing strains. This observation suggested that brown necrosis follows rust infection that takes place at the time of heading or a few days thereafter. An experiment was therefore conducted to determine whether such is the case (table 2). Heads and necks of Hope and Tenmarq in four different stages of development were inoculated with stem rust. The four stages were (1) early boot, (2) late boot, (3) early heading, and (4) about 10 days after heading. In the early and late boot stages, the

inoculations were made by hypodermic injections into the boot. In the two later stages, they were made by wrapping the heads and necks in sterilized gauze saturated with a suspension of rust spores in boiled distilled water. A wrapping of cellophane was then placed over the gauze to prevent drying. The heads were unwrapped after 18 hours. The rust inoculum used, which was the same in all cases, was collected in the field from three of the first infection centers that developed in the agronomy plots at College Station and was identified by pathologists at the University of Minnesota as races 11 and 17. To this was added inoculum of physiologic race 21 from greenhouse cultures. Hope wheat, in the seedling stage, is susceptible to all three of these races. The results of these inoculations are shown in table 2.

TABLE 2.—*Effect of inoculating Hope and Tenmarq wheat with a composite inoculum of Puccinia graminis tritici, races 11, 17, and 21, at different stages of development*

Variety	Stage of host at time of inoculation	Reaction	
		Rust	Brown necrosis
Hope	Early boot	Heavy	None.[1]
Tenmarq		do	Do.
Hope	Late boot	Trace	Heavy.
Tenmarq		Heavy	None.
Hope	Early head	None	Light.
Tenmarq		Heavy	None.
Hope	Late head (about 10 days after heading).	None	Do.
Tenmarq		Heavy	Do.

[1] No brown necrosis at 15 days but a heavy development on the spikes and necks about 5 days later.

A heavy infection of stem rust, but no brown necrosis, developed in Tenmarq from inoculations at all four stages.

A heavy development of rust on the necks, glumes, and awns of Hope appeared by the fifteenth day after inoculating in the early boot stage, but no brown necrosis appeared then. However, about 5 days later the rust pustules were surrounded by brown necrotic areas and development of rust pustules ceased.

In Hope plants inoculated in the late boot stage, a trace of small rust pustules began to appear on some of the glumes of the lower spikelets 12 days later. On the thirteenth day brown necrosis started developing around these pustules, after which there was no further development of pustules, and the brown necrosis continued developing for 2 days until it extended over the necks, glumes, rachises, and awns.

No rust pustules appeared on Hope plants inoculated in the early heading stage, but a light development of brown necrosis appeared on about the tenth day after inoculating and by the twelfth day the development was fairly heavy.

Where Hope was inoculated about 10 days after heading, no stem rust, brown necrosis, or white necrotic flecks developed. The results of these inoculations show that brown necrosis in Hope wheat developed, after an exposure to stem rust infection, while the host tissues were still succulent and comparatively young and before the maximum of mature-plant resistance to the rust had been acquired.

REACTION TO STEM RUST IN VARIETIES HAVING DIFFERENT TYPES OF RESISTANCE

An experiment was conducted to determine (1) whether other varieties carrying the Hope type of resistance to stem rust would produce a like development of brown necrosis and (2) whether varieties having other types of resistance would give the same or different reactions. This experiment was conducted in a small plot at some distance from other wheats in which natural infection by stem rust did not occur until about 20 days after the artificial inoculations were made.

The wheat varieties in this experiment were Hope and Hope × Reliance (N. N. 1131), which carry a distinct mature-plant type of resistance derived from emmer; Kota × Webster (H–151) and Kota × Webster (H–231), which carry a distinct morphologic type of resistance (also of a mature-plant nature) associated with a long incubation period; Kanred and Thatcher, which carry a high degree of seedling resistance to several physiologic races of stem rust; *Triticum timopheevi* Zhuk., which carries a high degree of both seedling and mature-plant resistance; and Denton, Mediterranean (T. S. 3015–105), Kawvale, Cheyenne, and Quivira, which probably are susceptible to most physiologic races of stem rust in both the seedling and mature-plant stages.

Three plants of each of the above varieties were inoculated hypodermically while in the late boot stage of development, part of the inoculum being injected between the stem and sheath of the second leaf from the top of the plant and part into the boot. The inoculum used was from the same sources as in the previous experiments. On the fifteenth day after inoculating, the results were recorded (table 3).

After these notes had been made, the plants were observed carefully at intervals of about 5 days or less throughout the filling and ripening periods, and the changes that occurred are shown in footnotes in table 3.

TABLE 3.—*Effect of inoculating varieties of wheat with composite inoculum of Puccinia graminis tritici, races 11, 17, and 21, at the late boot stage of development*

Rust-reaction class	Variety	Reaction 15 days after inoculation	
		Rust pustules	Brown necrosis
Photologic resistance [1]	Hope	None	Heavy.
	Hope × Reliance (N. N. 1131)	do	Do.
Morphologic resistance.	Kota × Webster (H–151)	Heavy (small pustules)	None.
	Kota × Webster (H–231)	do	Do.
Seedling resistance	Kanred	Light (medium size)	Do.
	Thatcher	None [2]	Do.[3]
Seedling and mature-plant resistance.	*Triticum timopheevi*	do	Do.[4]
Susceptibility	Denton	Heavy	Do.
	Mediterranean (T. S. 3013–105)	do	Do.
	Kawvale	do	Do.
	Cheyenne	do	Do.
	Quivira	do	Do.

[1] See p. 817.

[2] A fairly heavy infection of stem rust on both inoculated and uninoculated culms developed about 35 days after inoculation.

[3] A small brown blotch developed below 1 of the nodes and another on 1 glume of 1 of the inoculated culms of Thatcher, but these were not typical brown necrosis.

[4] Smoky-appearing areas (not brown necrosis) developed on the leaf sheaths just above the basal swellings. These also developed on uninoculated culms later.

In the Hope and Hope × Reliance wheats some rust pustules appeared beneath the sheaths of the inoculated plants where the tissues were not exposed to direct sunlight. Brown necrosis did not develop beneath the sheaths in either the inoculated or the uninoculated plants. Brown necrosis started developing on the checks about 25 days after the inoculations were made, or 10 days after the first appearance of rust pustules on the inoculated susceptible varieties.

In this experiment and in those recorded previously the typical brown necrosis occurred only in Hope wheat and in some related strains having a similar type of mature-plant resistance to stem rust.

BROWN NECROSIS AS AN INDICATOR OF MATURE-PLANT RESISTANCE

In wheat breeding, particularly in backcrossing, it is important to be able to determine, prior to the blooming period, which plants carry the factor for mature-plant resistance to stem rust. This cannot be determined by seedling reactions. Eighty-one plants of F_2 generation of an H–44 × Marquis cross were inoculated hypodermically with a mixture of rust races 11, 17, and 21 just before coming into head. Four of these plants later developed root rot and were discarded. Fifty-four of the seventy-seven remaining plants developed brown necrosis within 15 days and were later found to carry a high degree of mature-plant resistance to stem rust. Eighteen other plants proved to be completely susceptible to stem rust and developed no brown necrosis. The five remaining plants showed a moderate degree of mature-plant resistance to stem rust of a morphologic type resembling that found in the Kota × Webster selections H–151 and H–231. These five plants were also entirely free from brown necrosis. The data are given in table 4.

TABLE 4.—*Reaction of F_2 plants of an H–44 × Marquis cross to hypodermic inoculations, at the late boot stage, with a composite inoculum of Puccinia graminis tritici, races 11, 17, and 21*

Reaction of plants to brown necrosis	Total plants	Plants showing indicated reaction to stem rust		
		Photologic resistance	Susceptibility	Morphologic resistance
	Number	*Number*	*Number*	*Number*
Necrotic	54	54	0	0
Not necrotic	23	0	18	5
Total	77	54	18	5

The fact that the photologic type of resistance, associated with brown necrosis, occurred in 54 plants out of a total of 77 suggests that it is inherited as a simple dominant. The inheritance of the morphologic type of mature-plant resistance, which occurred in five plants, could not be determined from the meager data. The type of mature-plant resistance, which amounts to near immunity, was in all cases associated with brown necrosis. In most cases it was found that this identification could be made before the later heads had reached the blooming stage, and hence in sufficient time to allow for crossing.

DISCUSSION

The experiments and observations presented herein show that under certain conditions dark-pigmented areas, to which the term "brown necrosis" is applied, may develop on the internodes, peduncles, glumes, and rachises of certain rust-resistant varieties of wheat following inoculation with *Puccinia graminis tritici*. This reaction appears to be confined to wheats having a specific type of mature-plant resistance, such as Hope, H–44, and certain other varieties. Since it has been found by Hart and Zaleski (*7*) that Hope wheat has a type of resistance dependent on high light intensity, and by the present writer that brown necrosis as well as one type of resistance is dependent on high light intensity, it is suggested that the term "photologic resistance" be applied to this type of resistance, which is very likely of a physiologic nature. The other type of resistance that segregates out of H–44 crosses appears to be of a "morphologic" nature as described by Hursh (*9*) and Hart (*6*). In the experiments and observations presented here, brown necrosis appeared when the tissues of the host were inoculated with stem rust spores before photologic resistance was fully developed.

Brown necrosis bears a resemblance to the lesions produced by several other disease organisms, particularly *Bacterium translucens* var. *undulosum*. It appears from an examination of the literature that brown necrosis has been confused with the symptoms of other diseases by the writer [6] (*10*) as well as by others. The writer's report [6] of having broken the association between resistance to stem rust and susceptibility to the so-called black chaff was based on a study of F_2 plants and F_3 lines from natural crosses between certain Kota × Webster selections and sister strains of Hope and H–44. From unpublished data, it now appears that the Kota × Webster selections carry a factor that is either epistatic to, or inhibits the development of, the photologic type of resistance. Consequently, this type of resistance is expressed in only approximately one-sixteenth of the F_2 plants of such crosses. All of the other resistant plants of the F_2 generation of such crosses owe their resistance to factors other than the factor for photologic resistance. The type of resistance found in the Kota × Webster selections, as well as one type that segregates out of H–44 crosses, is not associated with brown necrosis. Therefore, a large majority of the rust-resistant segregates from such crosses are free from brown necrosis. This type of segregation would seem to account for the belief that the association between resistance to stem rust and susceptibility to black chaff (brown necrosis) is not complete.

The field experiments reported by Waldron (*12*), Ausemus (*1*), Hayes et al. (*8*), and Bamberg (*2*), in which many of the hybrid strains having the Hope type of resistance appeared to be very susceptible to black chaff, were conducted in nurseries where stem rust was present and where brown necrosis might thus be expected to appear. Dusting with sulphur, which controlled an unidentified disease called black chaff in rust-resistant wheats in experiments by Greaney (*3*, *4*), also should control rust and prevent accompanying brown necrosis. The description and illustrations of the "internodal melanism," "which seemed to be of non-parasitic origin," reported by Hagborg (*5*) in derivatives of H–44 and Pentad wheats are typical of brown necrosis.

[6] McFADDEN, E. S. See footnote 3.

The confusion of brown necrosis with other diseases appears to have been universal. Although several investigators appear to have entertained doubts concerning the specific organism that caused the discoloration, most of them seem to have held the opinion that it was a disease of bacterial origin.

The inoculation experiments and observations reported herein suggest that brown necrosis might be expected to appear in epidemic proportions under field conditions when wheat varieties carrying the factor for potential photologic resistance to stem rust are exposed to heavy infections by stem rust spores while the spikes are emerging and the stems still elongating. Under normal field conditions in the spring wheat areas of the United States and Canada, inoculum seldom would be sufficiently abundant at that stage to produce a heavy development of brown necrosis unless the rust-resistant variety were grown in close proximity to an earlier maturing, rust-susceptible variety. It appears, therefore, that the fears repeatedly expressed by various workers that the black chaff disease of Hope and H–44 might become of a serious nature should wheats derived from these varieties become widely grown are entirely ungrounded. On the contrary, it would be expected that should wheats having the photologic type of resistance to stem rust become widely grown stem rust inoculum would be greatly diminished, and in consequence, the so-called black chaff disease (brown necrosis) would practically disappear.

SUMMARY

When varieties of wheat such as Hope and H–44, which have a specific type of mature-plant resistance to stem rust, were artificially inoculated with stem rust spores, or exposed to natural infection, dark-brown necrotic areas were produced around the points of infection. The term "brown necrosis" is suggested for this discoloration to distinguish it from several diseases of wheat which it resembles.

A critical review of the literature indicates that brown necrosis has often been confused with several wheat diseases, usually the bacterial black chaff disease caused by *Bacterium translucens* var. *undulosum*.

Two distinct types of mature-plant resistance to stem rust were found in segregates from an H–44 × Marquis wheat cross. These types are designated as "photologic" and "morphologic" resistance, the former being so termed because it is expressed only under high light intensity. Brown necrosis occurred only in plants carrying the photologic type of resistance, which offers a possible explanation of previous reports of close but incomplete "linkages" and "associations" between resistance to stem rust and susceptibility to the so-called black chaff.

Inoculation experiments with F_2 plants from a cross between H–44 and Marquis wheats suggest the feasibility of using the brown necrosis reaction as an indicator for identifying plants that carry the factor for photologic resistance at an early stage of their development. Since this determination can be made prior to blooming, it would simplify breeding for rust resistance by the backcross method.

LITERATURE CITED

(1) AUSEMUS, ELMER R.
1934. CORRELATED INHERITANCE OF REACTION TO DISEASES AND OF CERTAIN BOTANICAL CHARACTERS IN TRIANGULAR WHEAT CROSSES. Jour. Agr. Research 48: 31–57, illus.

(2) BAMBERG, R. H.
1936. BLACK CHAFF DISEASE OF WHEAT. Jour. Agr. Research 52: 397–417.

(3) GREANEY, F. J.
1931. SULPHUR DUSTING FOR THE PREVENTION OF A BACTERIAL DISEASE OF WHEAT CALLED BLACK CHAFF. Sci. Agr. 11: 274–280, illus.

(4) ———
1934. STUDIES IN CEREAL DISEASES XI: THE PREVENTION OF CEREAL RUSTS BY THE USE OF FUNGICIDAL DUSTS. Canada Dept. Agr. Bull. 171 (n. s.), 90 pp., illus.

(5) HAGBORG, W. A. F.
1936. BLACK CHAFF, A COMPOSITE DISEASE. Canad. Jour. Research 14 (C, Bot. Sci.): 347–359, illus.

(6) HART, HELEN.
1931. MORPHOLOGIC AND PHYSIOLOGIC STUDIES ON STEM-RUST RESISTANCE IN CEREALS. U. S. Dept. Agr. Tech. Bull. 266, 76 pp., illus.

(7) ——— and ZALESKI, KAROL.
1935. THE EFFECT OF LIGHT INTENSITY AND TEMPERATURE ON INFECTION OF HOPE WHEAT BY PUCCINIA GRAMINIS TRITICI. Phytopathology 25: 1041–1066, illus.

(8) HAYES, H. K., AUSEMUS, E. R., STAKMAN, E. C., and BAMBERG, R. H.
1934. CORRELATED INHERITANCE OF REACTION TO STEM RUST, LEAF RUST, BUNT, AND BLACK CHAFF IN SPRING-WHEAT CROSSES. Jour. Agr. Research 48: 59–66.

(9) HURSH, C. R.
1924. MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE RESISTANCE OF WHEAT TO PUCCINIA GRAMINIS TRITICI ERIKSS. AND HENN. Jour. Agr. Research 27: 381–412, illus.

(10) MCFADDEN, EDGAR S.
1930. A SUCCESSFUL TRANSFER OF EMMER CHARACTERS TO VULGARE WHEAT. Jour. Amer. Soc. Agron. 22: 1020–1034.

(11) ———
1937. FALSE "BLACK CHAFF" OF WHEAT PRODUCED BY INOCULATING WITH STEM RUST. (Phytopath. Note) Phytopathology 27: 801.

(12) WALDRON, L. R.
1929. THE RELATIONSHIP OF BLACK CHAFF DISEASE OF WHEAT TO CERTAIN PHYSICAL AND PATHOLOGICAL CHARACTERS. Science 70: 268.

(13) WILSON, GUY WEST.
1914. THE IDENTITY OF THE ANTHRACNOSE OF GRASSES IN THE UNITED STATES. Phytopathology 4: [106]–112.

ROOT COMPOSITION AND TOP DEVELOPMENT IN LARGE PECAN TREES HEADED TO VARIOUS DEGREES OF SEVERITY IN TOP WORKING[1]

By C. L. SMITH, *associate physiologist*, J. HAMILTON, *junior pomologist*, C. J. B. THOR, formerly *agent*, and L. D. ROMBERG, *agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

An important phase of pecan (*Carya pecan* (Marsh.) Engl. and Graebn.) orchard development and management is the top working of native or undesirable trees to desirable varieties. Top working is practiced on trees of all sizes but is usually confined to trees having trunks less than 12 inches in diameter. Many different methods of top working pecan trees have been practiced, but not all successfully. In many instances the heading back of the top has been severe; the large wounds made have failed to heal and have become badly infected with wood-decaying fungi, with the result that the tops have been only partially rebuilt. The main incentive for severe topping is that it requires fewer buds or grafts and less time and expense to top-work a tree when it is cut back heavily than when it is cut back less severely. To be satisfactory, a method of top working must be biologically successful, i. e., the tree should be rebuilt to normal size and vigor; furthermore, the cost must be low.

The experiment herein reported was designed to determine the effects of different degrees of heading large pecan trees on their subsequent development after being top-worked to desired varieties.

MATERIAL AND METHODS

EXPERIMENTAL BLOCK

The experimental block, consisting of 9 rows of 32 trees each, excepting a few spaces where the trees had previously died, was located in the orchard of H. G. Lucas, in the valley of the Pecan Bayou, near Brownwood, Tex. The trees were 43 years old at the beginning of the experiment, having been grown from orchard-planted seeds and seedling trees planted in 1888. The trees were 40 feet apart on the square system. The average diameter of the tree trunks was about 12 inches in March 1931.

DEGREES OF SEVERITY OF HEADING

No heading.—One row of trees was left as checks without being cut back or top-worked (fig. 1, *A*).

Headed to stumps.—The trees in two rows were cut off approximately 3½ feet above the ground (fig. 1, *B*). Sloping wounds were made with the exposed surface facing the north so as to prevent drying out as much as possible. These wounds were from 12 to 18 inches in diameter except on a few small trees.

Headed to first forks.—The trees in two rows were cut off 8 to 10 inches above the first forks (fig. 1, *C*). The wounds were made at

[1] Received for publication July 11, 1938.

FIGURE 1.—Typical trees headed to different degrees of severity: *A*, Check tree, no heading; *B*, headed back to stump; *C*, headed to first forks. Note large wounds 6 to 8 inches in diameter.

right angles to the branches at first, and later cut to a slope after new shoot growth was sufficiently advanced. These wounds averaged 6 to 8 inches in diameter.

Headed to second forks.—Trees in two rows were cut off about 8 or 10 inches above the second forks (fig. 2, *A*). The wounds were made and treated in the same manner as in the trees headed to the first forks. These wounds averaged about 6 inches in diameter.

Headed to third forks.—Alternate trees in two rows were headed just above the third forks (fig. 2, *B*). The wounds averaged about 3 or 4 inches in diameter and were made and treated as in the trees headed to first forks.

Headed to fourth forks.—Alternate trees in two rows were headed just above the fourth forks (fig. 2, *C*). The diameter of the cuts averaged 2½ to 4 inches, most of them being not over 3 inches, and were made and treated as in the trees headed to first forks.

SUBSEQUENT PROCEDURE

The heading was all done in March 1931, and all wounds were protected by pruning paint. About one-half of the trees were grafted in the spring of 1931; the others were budded in late summer of that year. Trunk-circumference measurements and yield records of all trees were taken each year. A record of the cost of top working the trees in each treatment was also kept, as well as notes on the healing of wounds and infection by wood-rot fungi. However, this paper is concerned principally with the seasonal composition of the roots of trees and the growth responses following the different degrees of severity of heading.

Root samples were obtained from trees under each treatment at intervals from October 2, 1931, to January 23, 1934, and were analyzed for dry matter, sugars, starch, hemicellulose, and nitrogen. Three samples from check trees were taken after January 23, 1934. The data for the composition of roots of the check trees indicate the nutritional condition of normal trees under the conditions of the experiment.

ANALYTICAL METHODS

Sampling

Roots as uniform as possible but ranging between 5 and 20 mm in diameter were taken from trees in each treatment. The roots from each tree were washed free of adhering soil, wiped with a cloth towel, and allowed to dry in the air for 30 minutes. A composite sample of approximately 300 g was then made from the lots of roots. After the ends from each length of root had been clipped off, the remaining portion was cut into thin sections with pruning shears, placed in a covered soil-moisture can, and thoroughly mixed by shaking.

Two tared glass-stoppered weighing bottles were filled from this composite sample, weighed before any appreciable moisture loss occurred, and then saved for the determination of dry matter.

Two 50-g samples were weighed and transferred to pint fruit jars containing 250 ml of boiling 95-percent ethyl alcohol and a small amount of calcium carbonate. The covers were placed loosely on the jars and the boiling was continued for half an hour, after which the jars were removed from the water bath and sealed while hot. With this material this procedure is calculated to give a sample preserved in approximately 80-percent alcohol by weight.

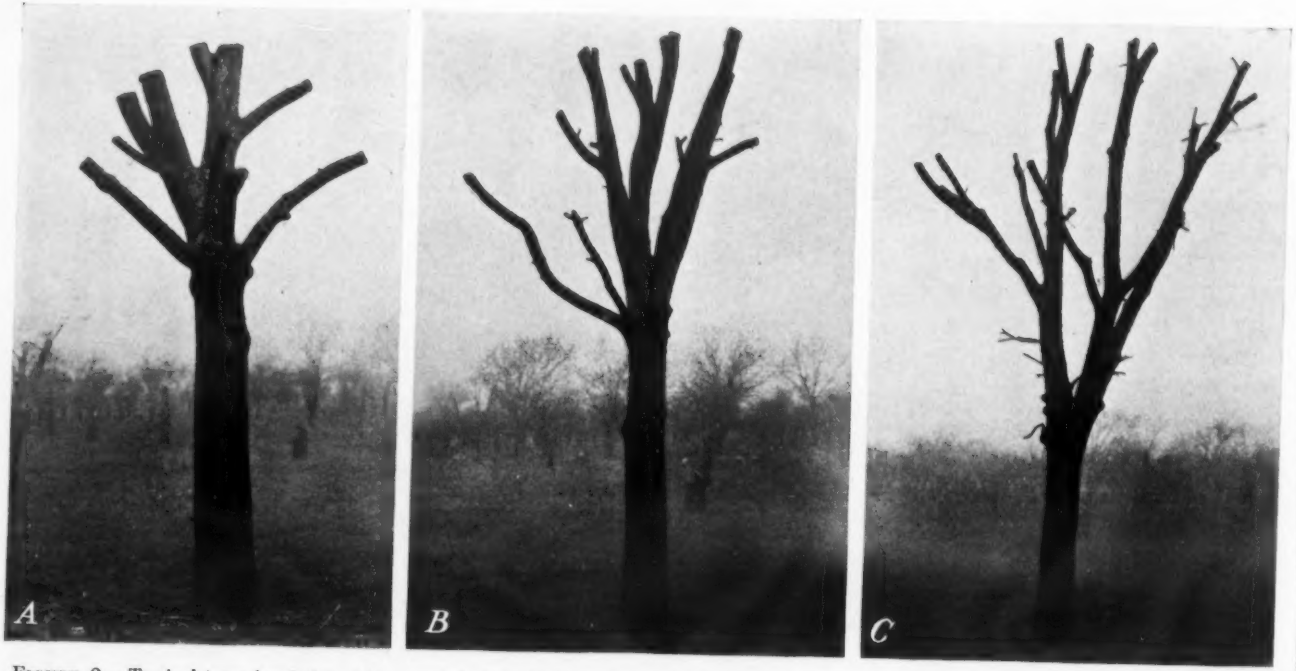


FIGURE 2.—Typical trees headed to (*A*) second forks, (*B*) third forks, (*C*) fourth forks. Note the small wounds, which heal easily, and the large framework for placing of buds and grafts.

Determination of Dry-Matter Content

The samples were dried in a vacuum oven at 80° C. and at a pressure of less than 1 inch of mercury. Since preliminary tests at 6, 12, 18, and 24 hours of drying showed that practically constant weight was attained in 6 hours, the samples were dried for two 6-hour periods and weighed at the end of each. The difference between these two weights was always well under 0.5 percent of the total moisture loss.

Determination of Sugars

Each alcohol-preserved sample was filtered and the solid portion transferred to an alundum extraction thimble 45 by 127 mm. It was then extracted for 24 hours in a large-size Soxhlet apparatus with the filtrate and alcoholic washings in the extraction flask. The residue was removed, dried in an air oven at 100° C. for 4 hours, and ground in a drug mill, care being taken that no significant loss of material occurred. The ground residue was again extracted for 24 hours with the same alcoholic solution. Preliminary tests had shown that the grinding and second extraction were necessary in order to insure complete extraction of sugars. The final residue was dried in an air oven at 100° for 4 hours, weighed, and saved for the determination of starch.

The alcoholic extract was concentrated in vacuo at a temperature below 55° C. to a volume of a few milliliters. This was taken up in water and filtered into a 250-ml volumetric flask. After adding 25 ml of saturated neutral lead acetate solution to the filtrate and washings, the mixture was made up to volume. The mixture was filtered through a dry filter, and the excess lead was precipitated from the clarified solution by means of anhydrous disodium phosphate. After being filtered through a dry filter, the solution was used for the determination of reducing and nonreducing sugars.

Reducing sugars were determined in 25-ml aliquots by the Munson-Walker method, the amount of reduced copper being determined by the volumetric permanganate method (*2, pp. 190–192* [*34–39*]).[2] Results were calculated as dextrose.

For nonreducing sugars, hydrolysis was effected with hydrochloric acid at room temperature by the procedure given in Official Methods (*2, p. 189* [*28*]). Results were calculated as sucrose.

Determination of Starch

The dried and weighed residue from the alcoholic extractions was ground once more in the drug mill, and samples were weighed from this for the individual starch determinations.

Starch was determined by direct acid hydrolysis as outlined in Official Methods (*2, p. 119* [*21*]) except that the preliminary washing with water was omitted since the material was already sugar-free and it was desired that any dextrin which might be present should be included in the result. The starch by the diastase method was subtracted from the starch by direct acid hydrolysis for corresponding samples, and the values thus obtained are referred to as hemicellulose. These values include the carbohydrate bodies that are not hydrolyzed

[2] Italic numbers in parentheses refer to Literature Cited, p. 841.

by diastase, such as dextrins and pentosans, but are hydrolyzed by dilute mineral acids.

Starch was also determined by the diastase method essentially as outlined in Official Methods (*2, p. 120* [*23*]). A freshly prepared 0.5-percent solution of takadiastase in water was used instead of the malt extract. The preliminary washing of the sample was again omitted for the same reasons as before.

In both cases the data for the starch content were finally calculated back to the original alcohol-preserved sample.

Determination of Total Nitrogen

The dried residue from the determination of dry matter was ground in a drug mill, and 2-g samples were weighed out for nitrogen determinations. Total organic nitrogen was determined by the Kjeldahl-Gunning-Arnold method as outlined in Official Methods (*2, p. 8* [*24*]). The analytical data are presented in figures 3 to 8.

EXPERIMENTAL RESULTS

CHEMICAL COMPOSITION OF ROOTS

Dry Matter

The percentage of dry matter in pecan roots fluctuated considerably during the course of the experiment (fig. 3). These fluctuations occurred at approximately the same time in roots of all trees but were less in roots of trees headed to stumps and first forks than in any of the others. The percentage of dry matter was apparently largely determined by the moisture supply in the soil and its absorption and storage in the roots, since in general the least dry matter was found in winter and spring when the soil moisture was relatively abundant. That the fluctuations were not due to a great extent to changes in amounts of the constituents of the dry matter is shown by the fact that starch increased at times when the percentage of dry matter was decreasing. The relatively high maximum concentration of starch and its wide fluctuation would have an appreciable effect on the dry-matter content, but not nearly so much as water absorption by the roots.

Sugars

The reducing-sugar content of roots of the trees in all treatments was relatively low throughout the experiment, but after May 1932 roots of the stumps contained less than any others. At the end of the experiment there was little difference in the amount of reducing sugars in any of the other treatments (fig. 4).

The nonreducing sugars behaved in a manner similar to the starch. There was a larger amount and greater fluctuation in the roots of the trees least severely headed (fig. 4). The fluctuations of nonreducing sugars were, in general, somewhat parallel to those of starch after May 1932. In the roots of trees headed to stumps the nonreducing sugars were low throughout the experiment, while in those headed to first and second forks there was an increase beginning about the middle of 1933 and continuing to late fall. In general, the nonreducing-sugar content in roots of trees headed to third and fourth forks was lower than in roots of check trees until near the end of the experi-

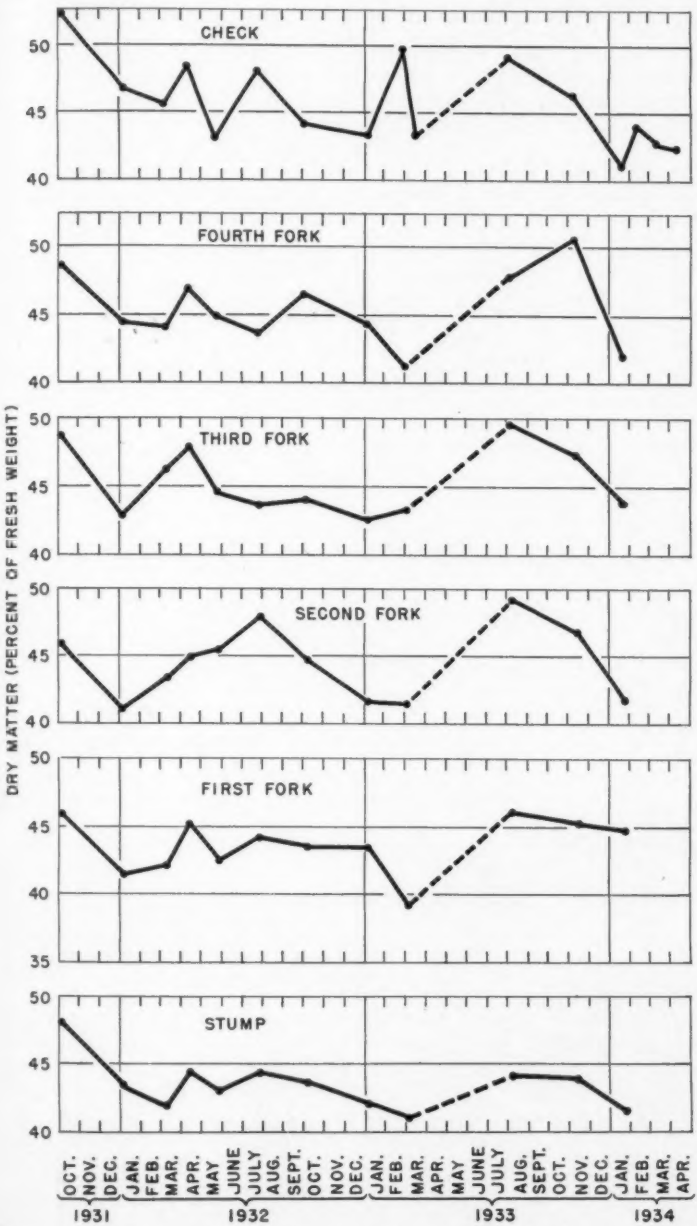


FIGURE 3.—Percentage of dry matter in roots of normal (check) trees and those headed to different degrees of severity in top working.

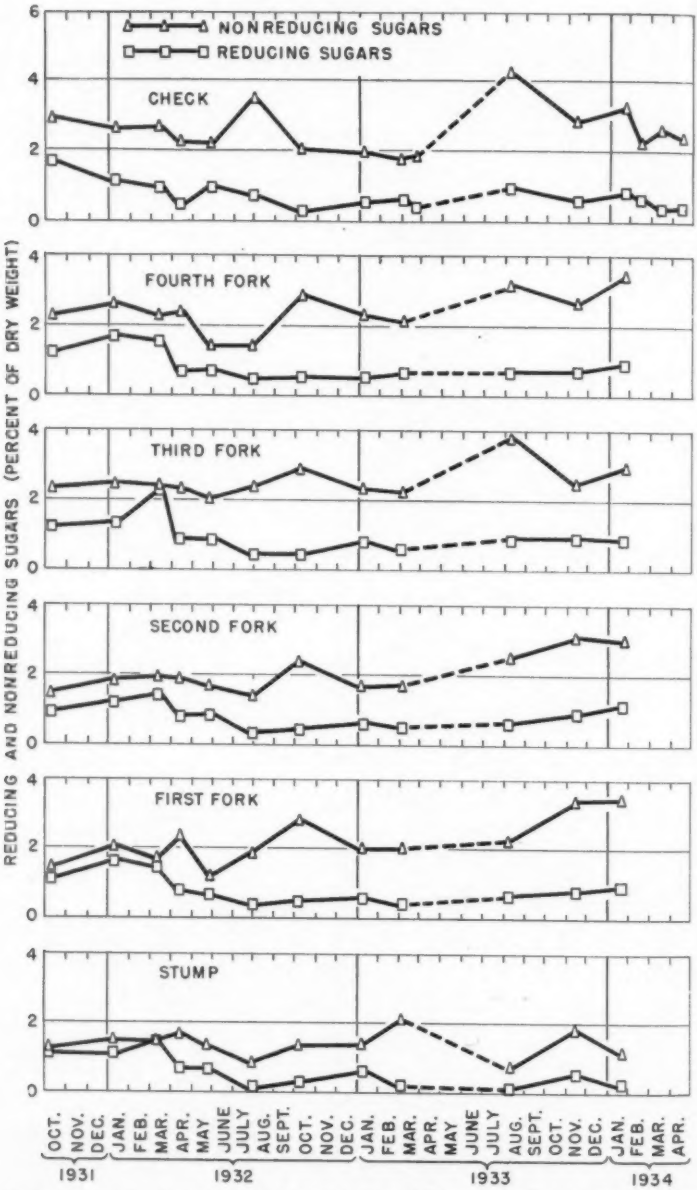


FIGURE 4.—Percentage of reducing and nonreducing sugars in roots of normal (check) trees and those headed to different degrees of severity in top working.

ment. There was also somewhat less fluctuation of the nonreducing sugars in roots of these trees than in the check trees. When the values for nonreducing sugars are calculated on a fresh-weight basis and then plotted, they form a curve that parallels somewhat the curve for dry matter (fig. 5). This shows that the concentration of these sugars varies to some extent with the variation in dry matter; however, calculations show that the change in the amount of dry matter was usually not proportional to the change in amount of nonreducing sugars. Therefore, a part of the variation in nonreducing sugars was due to actual increases or decreases of the total amount in the roots. The data are given for check trees only, since those for headed trees show similar variations.

Starch

The data for starch analyses are given in figure 6. The starch content in the roots of check trees showed pronounced fluctuations that seemed to be more or less seasonal in character. The root samples were not taken at sufficiently frequent intervals to determine the exact seasonal trends, but the data indicate that in general starch increases from a minimum in summer to a maximum in autumn, and then decreases to a minimum in summer again. This is in line with what LeClerc du Sablon (*7*), Murneek (*9*), and Davis (*4*) have found in various deciduous trees.

In 1931 the starch in the roots of check trees did not reach a maximum in the autumn but continued to increase through the winter to a maximum in March. In each of the other two seasons the starch concentration attained a maximum in autumn and then decreased during the winter. The only known differences in the conditions during 1931 as compared with 1932 and 1933 are that the nut crop of 1931 was much smaller and the leaves remained on the trees later in the fall.

With the initiation of growth in the spring of 1932 there was a rapid disappearance of starch from the roots; the starch was probably used in growth and blossom development. From the last of May to October 5 there was a gradual increase in the starch. This increase is probably due to storage of the excess assimilated from photosynthesis by the leaves. Little vegetative growth was being made at this time, and the nut crop had not begun to fill; the leaves were full-grown and probably capable of maximum photosynthesis.

The starch decreased during the nut-filling period, which was completed about the middle of November, and continued to decrease until the following March. The nut crop was large and made a heavy demand for carbohydrates for oil formation in the kernels (*11*), and the starch reserve was probably used to supplement the carbohydrates produced by photosynthesis. Defoliation occurred shortly after the nuts were harvested, and therefore the starch reserve could not be built up again by photosynthetic activity. It will be noted that there was also a rapid decrease in starch after leaf fall in 1933, which seems to corroborate the view that there is usually a winter decrease of starch in roots of deciduous trees caused by its translocation, in the form of simpler carbohydrates, from the roots to the aerial portions of the trees.

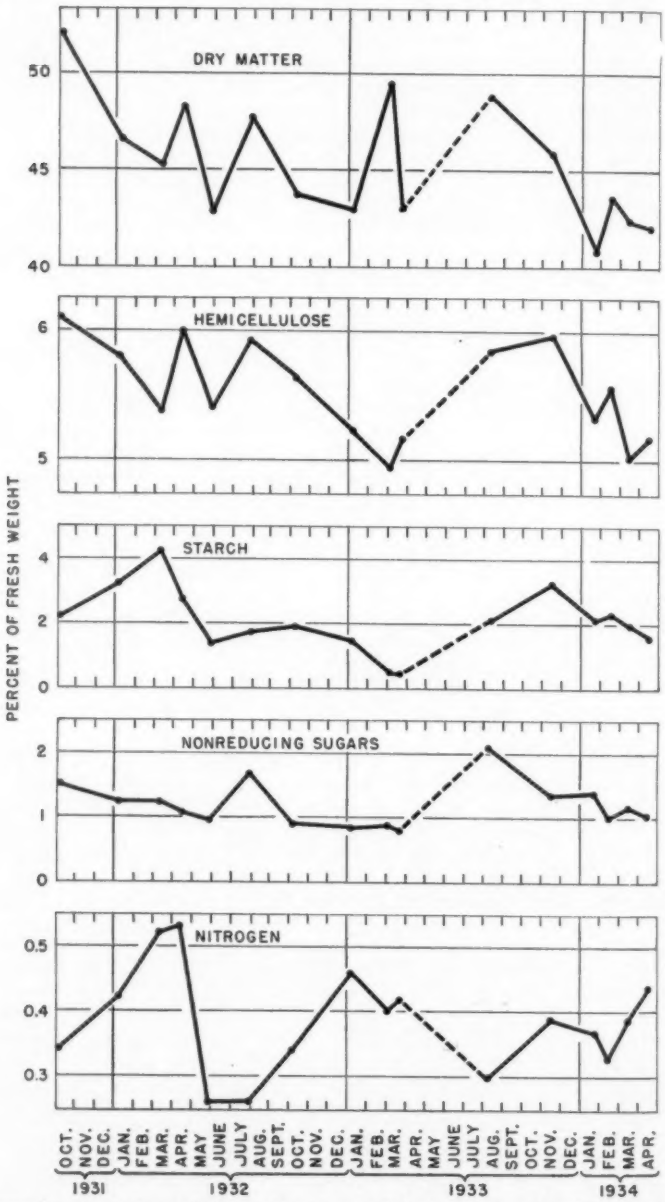


FIGURE 5.—Percentage of dry matter, hemicellulose, starch, nonreducing sugars, and nitrogen on a fresh-weight basis in roots of normal (check) trees. Note that the concentration of hemicellulose and nonreducing sugars varies to a considerable extent with the variation in dry matter.

From March to November 1933, when the nut crop was light and the conditions in late summer and fall were poor for vegetative growth, the starch content increased to a relatively high level and then decreased as in the season of 1932.

Although the data shown in figure 6 were calculated on a dry-weight basis, the same type of curve is obtained by plotting the amounts of starch in roots of the check trees calculated on the fresh-weight basis (fig. 5). A comparison of this curve with the one for dry matter

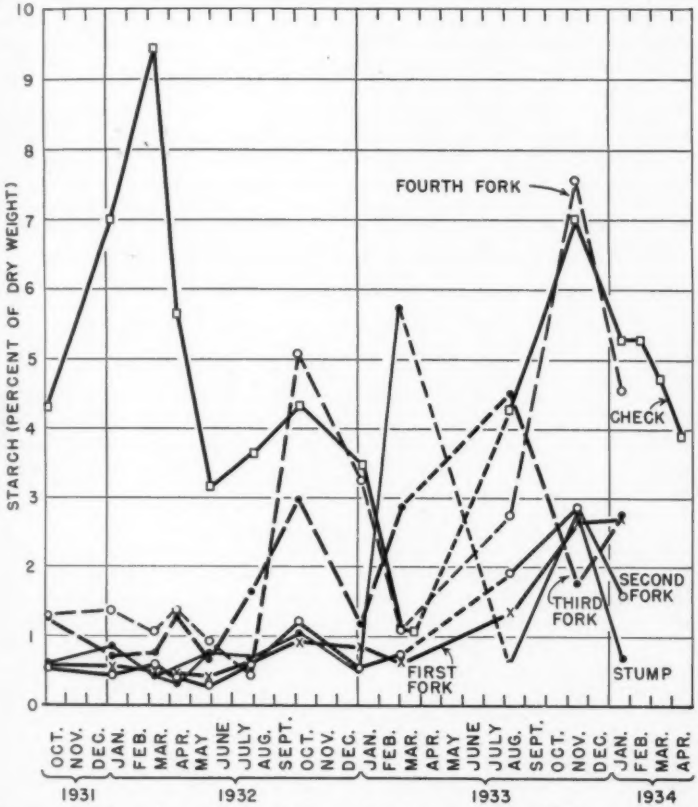


FIGURE 6.—Percentage of starch in roots of normal (check) trees and those headed to different degrees of severity in top working.

indicates that the starch fluctuations are largely independent of dry-matter variations and to a great extent are caused by actual increases or decreases in the total amount of starch in the roots. In fact it would be impossible for the changes in amount of dry matter to cause the starch fluctuations in most cases because the starch content on a fresh-weight basis increases with decreases in dry matter. The reverse is also true, and the proportionate variations in the two constituents are not the same.

The starch content in the roots of all the cut-back trees was very low from October 1931 to the middle of the summer of 1932, and showed relatively little fluctuation regardless of the degree to which the trees were cut back. Subsequent to August 1932, however, there was a progressive increase in the amount of starch in the roots of trees under the third- and fourth-fork treatments. In the roots of trees headed to third forks the starch began to increase during the summer of 1932, and it fluctuated in the same manner as that in check trees but to a lesser degree. The starch content in roots of fourth-fork trees started to increase at the same time and during the latter part of the experiment was as high as that in roots of the check trees. Its fluctuation was also very similar to that in the roots of check trees, in both manner and degree, during this period.

The amount of starch and its fluctuations in roots of first- and second-fork trees were very similar throughout the experiment. There was no progressive increase in starch content until the summer of 1933, and at the end of the experiment it was still relatively low. The starch content of the roots of trees cut to stumps was low throughout the experiment, except for one abnormally high concentration on March 3, 1933. The reason for this large value is not known.

Hemicellulose

The average amount of hemicellulose in roots of the trees under the different treatments was about 12 percent on a dry-weight basis and showed no consistent seasonal variations (fig. 7). However, on a fresh-weight basis the hemicellulose varied with the dry matter (fig. 5), indicating that the changes in the hemicellulose values are due largely to changes in dry matter rather than to changes in the total amount in the roots. Therefore, it is apparent that the hemicellulose was not utilized as a reserve carbohydrate to any appreciable extent.

Nitrogen

The amount of nitrogen in the roots of trees headed to stumps was, in general, somewhat larger than in the roots of the trees under any other treatment, but it showed practically no seasonal fluctuation (fig. 8). In roots of trees headed to first and second forks the nitrogen content showed somewhat greater fluctuations, which appeared to be seasonal except that there was a continued decrease in the amount of nitrogen after March 3, 1933, to the end of the experiment. In the roots of trees headed to third forks the amount of nitrogen was as low as or lower than that in roots of second-fork trees, but it showed more definite seasonal fluctuations. The nitrogen increased from October 1931 to April 12, 1932, after which it decreased to July 27. Then there was a gradual increase to March 3, 1933, followed by a decrease to August 9, and then an increase again to January 23, 1934.

The nitrogen content in roots of trees headed to fourth forks was a little higher as a rule than in roots of third-fork trees and showed greater seasonal fluctuations, which coincided with those in the trees under third-fork treatment. In roots of the check trees was found the greatest seasonal variation in nitrogen. The fluctuations occurred at the same time as those in the third- and fourth-fork trees but were wider over the period of the experiment.

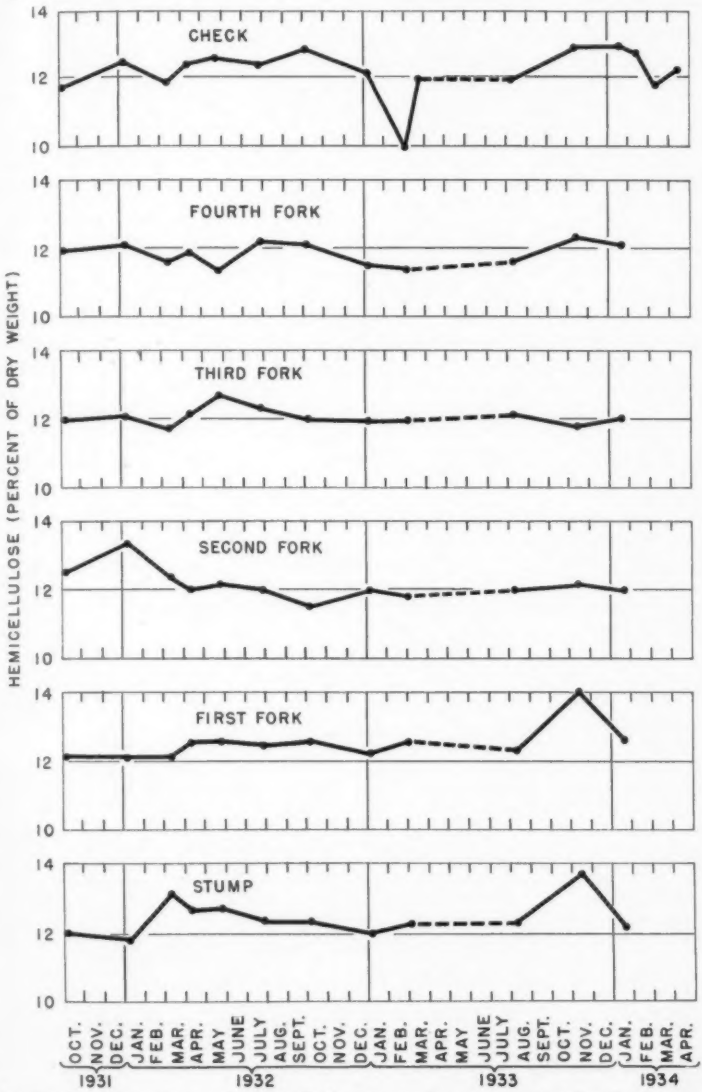


FIGURE 7.—Percentage of hemicellulose in roots of normal (check) trees and those headed to different degrees of severity in top working.

A comparison of the dry-matter and nitrogen curves in figure 5 shows that the nitrogen fluctuation was independent of the variations in dry-matter content. In general, nitrogen increased on both the fresh-weight and dry-weight bases as the dry matter decreased, and vice versa. It is probable that this was only coincidental, however, and that neither factor was the cause of the other.

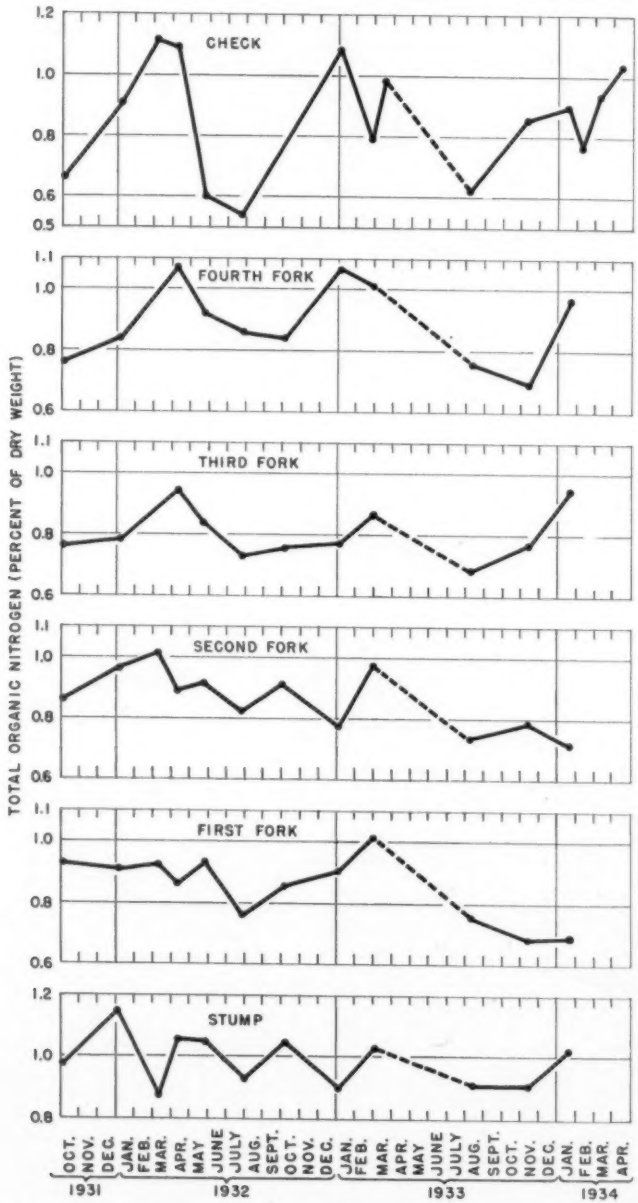

CHECK
FOURTH FORK
THIRD FORK
SECOND FORK
FIRST FORK
STUMP
TOTAL ORGANIC NITROGEN (PERCENT OF DRY WEIGHT)
OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MAR. APR.
1931 1932 1933 1934

FIGURE 8.—Percentage of total organic nitrogen in roots of normal (check) trees and those headed to different degrees of severity in top working.

TREE GROWTH

Gain in Cross-Sectional Area

The following data show the average increase from March 1931 to December 1933 in cross-sectional areas of the trunks of normal (check) trees and trees headed to different degrees of severity in top working.

Treatment:	*Square inches*[1]	Treatment:	*Square inches*
Normal (check) trees	22. 60	Second-fork trees	3. 98
Fourth-fork trees	5. 61	First-fork trees	3. 53
Third-fork trees	6. 34		

[1] Average increase in cross-sectional area of trunks.

This increase is greater the less severe the heading treatment, except that the third-fork trees made somewhat more trunk growth than fourth-fork trees. The check trees show an increase in trunk growth more than three times as great as any of those cut back. No measurements are recorded for trees given the stump treatment, since most of these died to the ground before the end of the experiment.

Top Growth and Healing of Wounds

It is believed that the amount of top growth of cut-back trees is a much more accurate measure of their actual growth than the increase in cross-sectional area of the trunks. No measurements were made of the top growth of trees under the different treatments, but the relative

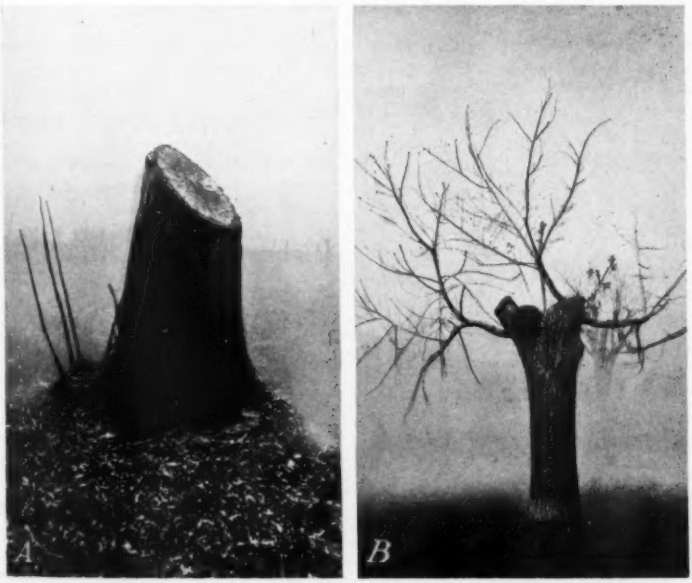


FIGURE 9.—Pecan trees cut back March 1931, headed to different degrees of severity. *A*, Headed to stump (photographed April 1932); grafts placed at the top were blown off by wind; stump dead to ground and sprouts coming from roots. *B*, Headed to first fork (photographed April 1934). Note that the large wounds have not started to heal.

amounts of this growth and the degree of healing of wounds are shown by photographs of typical trees near the end of the experiment (figs. 9 and 10).

All the large trees that were cut back to the stumps died to the ground, and sprouts came up from the roots (fig. 9, *A*). A few of the smaller stumps lived, but the wounds have made little progress in healing, and much difficulty has been experienced in preventing grafts and buds from being blown off by winds.

The first-fork trees have made relatively little top growth, and the large wounds have not begun to heal (fig. 9, *B*). Many of the new branches that grew on these trees were blown off by winds, since the unions with the large limbs were not strong.

Trees in the second-fork treatment made considerably more top growth than first-fork trees, but the wounds have made little progress in healing (fig. 10, *A*). Some of the new branches on these trees were also blown off by winds.

The top growth in third-fork trees was much greater than in second-fork trees, and the wounds are partially healed (fig. 10, *B*).

The fourth-fork trees made more top growth than any other cut-back trees, and the wounds are practically healed (fig. 10, *C*). The tops are now reestablished to such an extent that the trees can bear large crops of nuts. No loss of buds or grafts from windstorms occurred in these trees or in those cut back to third forks.

DISCUSSION

The data show certain definite differences in the composition of roots of check trees as compared with those of trees that were cut back in top working. These differences were nearly the same for all cut-back trees during the first season but tended to disappear as the tops were reestablished.

Starch and nitrogen were the largest variables of the constituents determined, and their fluctuations seemed to be more or less seasonal. The data for starch seem to indicate a summer minimum and a fall maximum, with a small winter decrease following leaf fall and a rapid decrease during spring growth and blossoming. In the fall of 1931, however, the starch in the roots of check trees increased from October until the following March, while in 1932 and 1933 it reached a maximum in the autumn and then decreased during the winter. The nut crop of 1931 was very small, the conditions were poor for vegetative growth, and the foliage remained on the trees until late in the fall. Under these conditions the excess carbohydrates produced by photosynthesis were probably translocated to the roots and stored as starch. There may also have been some weather conditions prevalent during the period that were favorable for the continued translocation of carbohydrates from the aerial portions of the trees to the roots, whereas in the other two seasons such conditions were absent. Murneek and Logan (*10*), in a study of autumnal migration of nitrogen and carbohydrates in the apple tree, concluded that since starch and possibly other more complex carbohydrates are hydrolyzed to sugars with the onset of cold weather, or vice versa, modifications induced by weather often are of greater amplitude than possible seasonal trends.

The starch in the roots of check trees reached its lowest concentration in the spring following the large nut crop of 1932. The starch

FIGURE 10.—Pecan trees cut back March 1931 (photographed April 1934), headed to different degrees of severity. *A*, Headed to second forks; large wounds have made little progress in healing. *B*, Headed to third forks; note that the wounds are relatively small and are partially healed. *C*, Headed to fourth forks; the wounds are small and practically healed; the tops are large enough to bear good crops of nuts.

decreased rapidly during the nut-filling period and, no doubt, was used in oil formation in the kernels. Thor and Smith (*11*) have shown that there is a heavy demand for carbohydrates during the period of oil formation in the pecan nut in the early fall, and that most of this carbohydrate material is translocated to the nut during this period. On the basis of microscopic studies, Finch and Van Horn (*5*) concluded that starch storage in fruit-bearing shoots of the pecan was initiated in early summer, reached a maximum shortly before nut filling began, and then decreased rapidly during this period. A comparable decrease did not occur in nonfruiting shoots. Davis (*4*) found that the starch content in the roots of nonbearing sugar-prune trees was much higher than in roots of bearing trees. He also observed a very rapid decrease of starch in the bark, spurs, and wood of bearing trees during the period of ripening of the fruit, while during the same period there was not a comparable decrease in nonbearing trees. With the beginning of growth in the spring, there was a decrease in starch content of both bearing and nonbearing trees, which attained a minimum at blossoming, but the minimum in the bearing trees was much lower than in those not bearing. This probably explains the very low concentration of starch in the spring of 1933 following the large nut crop of 1932. The starch content of the roots was depleted during the nut-filling period; and, since the foliage dropped soon after the nuts were harvested, the starch was not built up again by photosynthesis. Consequently, with the normal winter decrease of starch and the initiation of growth in the spring, the starch had reached a very low level on March 3. No analyses were made during the period of shoot growth and blossoming in the spring of 1933, but it is likely that the starch continued to decrease during this period since the data for 1932 and 1934 show decided decreases in the starch during similar periods.

The starch was relatively very low in the roots of all headed trees and showed little fluctuation during the first part of the experiment, but there was a progressive increase in its amount and fluctuation during the latter part of the experiment. Therefore, it seems evident that the quantity of starch is controlled largely by the growth and fruiting of the tree. In a study of the relation of leaf area and position to quality of fruit and to bud differentiation in apples, Haller and Magness (*6*) found an increase in blossom-bud formation and in size of fruit with an increase in leaf area on ringed branches, thus indicating that sufficient synthesis of food materials for blossom-bud differentiation and fruit development is obtained only with a relatively large leaf area. No doubt a similar condition prevails in the pecan tree, since the data seem to corroborate this view. During the first season after the trees were cut back the leaf area per tree was small under all treatments. The amount of leaf area was practically inversely proportional to the degree of severity of heading, but in no case was it adequate to synthesize sufficient carbohydrates to support growth, heal the wounds, and have much excess for storage in the roots. A considerable number of the leaves that grew on the new branches during the first season were removed when the top working was done in order to force out the buds and grafts. Thus there was actually less difference in the leaf area of trees in the different treatments during this period than if no leaves had been removed, and

consequently there was very little difference in the amount and fluctuation of starch.

During the latter part of the experiment much of the leaf area of trees under the stump, first-fork, and second-fork treatments was lost because the branches that grew from buds or grafts were blown off by wind. The loss of these branches also necessitated rebudding the trees and removing still more leaf area to force the buds. This partly accounts for the low starch values in the trees under these treatments at the end of the experiment as compared with those in the check trees, or those under the third- and fourth-fork treatments. The trees headed to third and fourth forks bore more branches and leaves than the severely cut-back trees, and practically none were blown off by winds. Therefore, toward the latter part of the experiment the tops of these trees had been reestablished to such an extent that the amounts of starch and its fluctuation in the roots were very similar to those in the check trees.

The data indicate that the sugars in pecan roots are not storage but labile forms into which other more complex carbohydrates may be converted for use or translocation. The amount of reducing sugars was relatively low in the roots of trees under all treatments, but after May 1932 the roots of stumps contained less than any others. The concentration and fluctuation of nonreducing sugars were greater in the roots of check trees and those least severely headed, the fluctuations being somewhat parallel to that of starch during the latter part of the experiment. The larger amount of sugars in roots of the check trees and those not severely headed, as compared with those that were, no doubt is a direct result of the greater supply from the larger leaf area, and the wider fluctuations may be due to the usage during growth and fruiting activities.

The concentration of nitrogen in the pecan roots was about the same for check trees and those cut back except that it was slightly higher in the roots of stumps. However, the extent of the seasonal fluctuations of the nitrogen was somewhat inversely proportional to the degree of severity of heading.

The nitrogen increased during the fall and winter and decreased during the spring, which indicates that it is used mainly in growth and blossom development. During late summer and fall when the vegetative growth has practically ceased it is assumed that the nitrogen continues to be absorbed and is stored. Weinberger and Cullinan (*12*) found that nitrogen was absorbed in large amounts by the roots of peach trees in late fall. They state (*12, p. 67*): "The roots of fertilized trees, however, practically doubled in nitrogen content in the same period [October 19 to December 22], compared to only a 15 per cent increase in check roots." Aldrich (*1*) obtained similar results with fall and winter applications of nitrogenous fertilizers to apple trees. Crane (*3*) has shown that fall applications of nitrogenous fertilizer are about as effective in increasing the growth and yield of pecan trees as are similar applications made in the spring. Loomis (*8*) presents data which indicate that nitrogen is rapidly synthesized to organic forms upon absorption by the roots. His data indicate that nitrogen is normally translocated in organic forms through the phloem, and that the process of digestion and translocation may be very rapid. In the bark of 3-year-old poplar trees one-half the nitro-

gen was digested and moved from the trunks in the first 4 weeks of the growing season. Murneek and Logan (*10*) found that there was an autumnal migration of nitrogen from the leaves to the stems prior to leaf fall in the apple. A part of the increase in nitrogen in the pecan roots occurring in the fall may have been due to the nitrogen that was translocated from the leaves prior to their abscission.

In the spring there is a large demand for nitrogen for the production of vegetative growth and blossoms, which probably cannot be met by absorption at that time. The nitrogen stored in the roots is probably translocated to other portions of the tree for these purposes, and the root content is thereby decreased. The fact that there was greater seasonal fluctuation in the amount of nitrogen in roots of the check trees and in those with less severe heading, where more growth and fruiting occurred, than in trees severely cut back, where little growth and fruiting took place, is further evidence that the explanation given above is valid.

According to the data the actual amount of nitrogen present at any one time could not be used as a measure of the physiological activity of the tree, since in the roots of trees headed to stumps the nitrogen present was generally as high as or higher than in the check trees. There was so little growth activity in the stumps that the root system was able to absorb nitrogen rapidly enough to supply this demand, and consequently the nitrogen remained nearly constant.

SUMMARY AND CONCLUSIONS

Pecan trees of an average diameter of about 12 inches were headed back in March 1931 to stumps, first forks, second forks, third forks, and fourth forks prior to being top-worked to improved varieties. Analyses of roots of these trees and of similar untreated ones were made at intervals from October 1931 to January 1934 for dry matter, reducing and nonreducing sugars, starch, hemicellulose, and organic nitrogen.

The dry-matter content of roots of pecan trees was higher in the summer and autumn than in the winter and spring, and apparently these fluctuations were controlled largely by transpiration and the moisture supply in the soil. The dry matter fluctuated less in roots of trees headed to stumps and first forks than in roots of check trees or those headed to second, third, and fourth forks.

Although the root samples were not taken at sufficiently frequent intervals to determine exact trends, the data indicate that starch increases from a minimum in summer to a maximum in fall or early winter, and then decreases again to a minimum in summer; also, that the fall maximum may be prevented in a heavy crop year because of the demand for carbohydrates for oil formation in the kernels of the nuts during filling.

The amount and fluctuation of starch in pecan roots appear to be largely dependent upon the amount of leaf area and the growth and fruiting of the trees. During the first year after the trees were cut back, the leaf area on all headed trees was relatively small, and the starch content was low and showed no seasonal variation. After this time, however, the amount of foliage showed an inverse relationship roughly proportional to the severity of heading back, and the amount of starch and its fluctuation were about proportional to the leaf area.

In the roots of check trees the starch content fluctuated widely, and its concentration was relatively high except in the winter and spring following the large nut crop of 1932.

The reducing-sugar content in the roots of all the trees was low and about equal throughout the experiment except in the stumps, where it was lowest during the last half of the experiment. The nonreducing sugars behaved in a manner similar to starch. The amounts were higher and there was more fluctuation as the leaf area per tree was increased. In general, the seasonal fluctuations of nonreducing sugars were nearly parallel to the starch variations but apparently these fluctuations were partly due to changes in the dry-matter content of the roots. The indications are that sugars in pecan roots are not storage but labile forms into which other more complex carbohydrates are converted for use or translocation.

There were only moderate fluctuations in the hemicellulose content in the roots of trees under the different treatments, and these variations were caused largely by changes in the dry-matter content of the roots.

The amount of nitrogen was somewhat higher in the roots of trees headed to stumps and fluctuated less than in those under any other treatment. In the roots of the check and of the third- and fourth-fork trees the amount of nitrogen fluctuated widely, increasing from late summer to early spring and then decreasing again, indicating that its main function is in growth and blossoming. In roots of first- and second-fork trees this same seasonal fluctuation occurred during the first 2 years of the experiment, but during the last year the nitrogen continued to decrease from March to January. The seasonal fluctuations in nitrogen concentration were independent of changes in dry-matter content of the roots.

For comparing the growth made by pecan trees during the first few years after they had been cut back in top working, the amount of top growth appears to be a better measure than the increase in the cross-sectional area of the trunk.

The rate of healing of wounds occasioned by the heading of trees was approximately proportional to the amount of leaf area per tree.

The data, together with observations made on the rate and facility of healing of wounds in the different treatments, indicate that, in heading pecan trees preparatory to top working, cuts more than 4 inches in diameter should be avoided. Larger cuts are hard to heal and, in heading trees severely enough to make wounds larger than 4 inches in diameter, the potential leaf area is automatically so reduced that it will probably be inadequate to synthesize sufficient foods for the normal recovery of the tree.

LITERATURE CITED

(1) ALDRICH, W. W.
1932. NITROGEN INTAKE AND TRANSLOCATION IN APPLE TREES FOLLOWING FALL, WINTER, AND SPRING SODIUM NITRATE APPLICATIONS. Amer. Soc. Hort. Sci. Proc. (1931) 28: 532–538.

(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. Compiled by the committee on editing methods of analysis. Revised to July 1, 1924. Ed. 2, 535 pp., illus. Washington, D. C.

(3) CRANE, H. L.
1932. TWO YEARS' RESULTS OF PRUNING BEARING PECAN TREES. Ga.-Fla. Pecan Growers Assoc. Proc. 26: 44–51.

(4) DAVIS, LUTHER D.
1931. SOME CARBOHYDRATE AND NITROGEN CONSTITUENTS OF ALTERNATE-BEARING SUGAR PRUNES ASSOCIATED WITH FRUIT BUD FORMATION. Hilgardia 5: 119–154, illus.
(5) FINCH, A. H., and VAN HORN, C. W.
1936. THE PHYSIOLOGY AND CONTROL OF PECAN NUT FILLING AND MATURITY. Ariz. Agr. Expt. Sta. Tech. Bull. 62, pp. [420]–472, illus.
(6) HALLER, M. H., and MAGNESS, J. R.
1933. RELATION OF LEAF AREA AND POSITION TO QUALITY OF FRUIT AND TO BUD DIFFERENTIATION IN APPLES. U. S. Dept. Agr. Tech. Bull. 338, 36 pp., illus.
(7) LECLERC DU SABLON.
1904–6. RECHERCHES PHYSIOLOGIQUES SUR LES MATIÈRES DE RÉSERVES DES ARBRES. Rev. Gén. Bot. 16: [341]–368, illus., 1904; 18: [5]–25, [82]–96, illus., 1906.
(8) LOOMIS, W. E.
1935. THE TRANSLOCATION OF NITROGEN IN WOODY PLANTS. Amer. Soc. Hort. Sci. Proc. (1934) 31 (v. 32): 61–64.
(9) MURNEEK, A. E.
1934. CARBOHYDRATE STORAGE IN APPLE TREES. Amer. Soc. Hort. Sci. Proc. (1933) 30: 319–321.
(10) ——— and LOGAN, J. C.
1932. AUTUMNAL MIGRATION OF NITROGEN AND CARBOHYDRATES IN THE APPLE TREE WITH SPECIAL REFERENCE TO LEAVES. Mo. Agr. Expt. Sta. Research Bull. 171, 30 pp., illus.
(11) THOR, CLIFFORD J. B., and SMITH, CHARLES L.
1935. A PHYSIOLOGICAL STUDY OF SEASONAL CHANGES IN THE COMPOSITION OF THE PECAN DURING FRUIT DEVELOPMENT. Jour. Agr. Research 50: 97–121, illus.
(12) WEINBERGER, J. H., and CULLINAN, F. P.
1935. NITROGEN INTAKE AND GROWTH RESPONSE IN PEACH TREES FOLLOWING FALL AND SPRING FERTILIZER APPLICATIONS. Amer. Soc. Hort. Sci. Proc. (1934) 31 (v. 32): 65–69.

STUDIES ON THE NATURE OF BROWN ROOT ROT OF TOBACCO AND OTHER PLANTS [1]

By JAMES JOHNSON [2]

Agent, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture, and *professor of horticulture, College of Agriculture, University of Wisconsin*

INTRODUCTION

Despite the highly specialized adaptation of roots to soil, it is surprising that decay of these organs is not more frequent, considering the varied environment to which they are exposed under intensive cropping. Normally it is only when a specific parasite is concerned that extensive root injury occurs on plants. When, therefore, a root necrosis develops frequently on a crop without any obvious parasite or unfavorable environment being present, it offers a problem of special interest to pathology. The brown root rot disease of tobacco (*Nicotiana tabacum* L.) appears to be a malady of this type, which has now been recognized for 20 years, particularly in the more northerly tobacco-growing States. The disease may affect a wide variety of plants, but evidently no serious economic losses from this cause have been reported to occur except on tobacco. Paradoxical as it may seem, the disease is favored by certain apparently nonsusceptible crops when used as preceding crops in rotations with tobacco, whereas the continuous culture of certain susceptible crops results in the gradual elimination of the causal agent of brown root rot from the soil. This conclusion, based upon many field experiments and observations over a 20-year period, together with repeated failure to isolate an organism that produces infection, has naturally led to reasonable doubt that a typical parasite is concerned.

It is not the object of the present paper to offer a new hypothesis to explain the disease, but rather to bring together the results of certain investigations undertaken for the purpose of securing further information regarding the nature of the causal agent. These relate chiefly to the general behavior and properties of the causal agent and the pathological histology of the disease. A brief summary of the results presented in this paper has been previously published (*9*) [3].

LITERATURE REVIEW

It is not deemed necessary in the present connection to consider the various ramifications of the problem in the literature relating to "soil toxins" and the injurious effect of preceding crops. The former has recently been reviewed by Loehwing (*13*), and the latter subject was treated by Garner et al. (*7*) in some detail in 1925. Toxic action on plant growth may be induced in artificial culture, and crop retardation

[1] Received for publication September 23, 1938.
[2] The writer is indebted to C. V. Kightlinger, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture, for help in selecting the soils used and for shipping the soils for experimental work to the Wisconsin laboratory.
[3] Italic numbers in parentheses refer to Literature Cited, p. 862.

may develop as a result of cropping systems in the total absence of root decay; the problems there involved are probably quite different from the brown root rot problem. Brown root rot, as far as can be judged by its behavior and symptoms, is a specific disease, although in the absence of a known causative agent it may not always be readily or definitely determined as such. The first detailed description of the malady was made in 1926 from this laboratory (*11*). In addition to the description of the disease, the failure to secure infection from the organisms isolated from diseased roots was emphasized. The destruction of the causal agent by heat and formalin sterilization as well as by desiccation of the soil was reported. Field experiments in the Connecticut Valley showed the strikingly injurious effect of certain preceding crops on succeeding crops of tobacco. The retarded growth was definitely associated with the presence of the brown root rot disease. Since 1926 continued field experiments of a similar type have been conducted in the Connecticut Valley and in Wisconsin, and these have yielded very similar results; consequently the data have not been published in detail (*10, 15*).

One line of investigation was developed further, however, by Thomas (*17*), who attacked the problem from the point of view of the chemistry of the soil. Thomas secured high degrees of depression of plant growth, accompanied by root discolorations resembling brown root rot, as a result of adding relatively pure forms of cellulose (filter paper) to the soil. The abundance of energy material furnished by the cellulose stimulated the growth of fungi to such an extent that a shortage of available nitrogen followed, resulting in depressed plant growth. More specifically, however, it was suggested that these normally saprophytic soil fungi, in their further search for nitrogen, invaded the root tissues of plants sufficiently to cause the necrosis known as brown root rot. This theory was later expanded by Mathews, Reneger, and Thomas (*14*), who attributed the disease to the specific action of *Rhizoctonia bataticola* under similarly favorable soil-environment conditions.

In the meantime other workers in the Connecticut Valley (*1, 4, 5, 6, 12, 16*) have leaned more directly toward the theory that a toxin or an unbalanced mineral nutrition may be the cause of brown root rot. Infusions of decomposing plant tissues, especially of timothy, for example, were found to depress plant growth as well as to cause discolorations and necroses of the roots resembling brown root rot. The decomposition of the plant tissues and the constituents of the infusions are naturally a result of microbial action and, although the toxicity of such infusions has been demonstrated, the results do not conclusively show that similar circumstances are responsible for the field conditions known as typical brown root rot. Nevertheless, until plant roots are treated with such toxins under aseptic conditions, some doubt may still exist as to the chemical explanation of the resulting necrosis.

That brown root rot is due to the presence rather than to the absence of some factor is indicated by its destruction by heat or antiseptics. It does not seem likely that the disease may be attributable to the lack of normal plant-food constituents in the soil. So far as known, no one has intensively studied the possible remote relation of a deficiency of the rarer elements to the disease.

MATERIALS AND METHODS

The soils used in these investigations were largely secured from known brown root rot fields in the Connecticut Valley, especially in the vicinity of Whately, Mass. Soils from other sections have been used for the laboratory work but in recent years have not given such heavy amounts of disease as have those from Massachusetts. The soils were stored in covered galvanized-iron cans or otherwise protected from drying out. The same lot of soil could thus be used over a period of at least 1 year without appreciable change in the amount of brown root rot present.

The experiments were largely performed during the winter months in the laboratory and greenhouse of the Wisconsin Agricultural Experiment Station. The tests for amounts of disease were made in 4-inch pots holding 500 to 600 gm. of soil, the trials usually being run in duplicate but repeated several times. At the end of about 4 weeks the roots were removed from the pots, washed free from soil, and floated out in water against a white background in order to estimate more readily the amount of disease present. Various means of recording the amount of disease on the roots have been tried, but the simple method of indicating different degrees of infection with plus signs appears to be quite satisfactory for the present purposes. The amount of disease present is, of course, generally expressed in the relative plant growth, but, owing to the erratic behavior of this character, it was not found to be reliable as a measure of the amount of disease. Tomatoes were most frequently used as test plants, thereby avoiding any danger of confusion with black root rot (*Thielaviopsis basicola* (B. and Br.) Zopf.), which is common in tobacco soils but to which tomatoes are immune.

Special methods or techniques are briefly referred to in this paper under the respective experiments where they were used.

EXPERIMENTAL RESULTS

BEHAVIOR OF THE CAUSAL AGENT

Brown root rot possesses many characteristics of a parasitic disease, although it shows little or no indication of being of a very infectious or rapidly developing type. That is, the time required for "infection" is relatively long, the spread of the disease through the tissue is rather slow, and the causal agent must be comparatively concentrated to produce much injury. Normally it requires 3 to 4 weeks to secure good symptoms (fig. 1) from brown root rot soil in greenhouse trials, and it is often difficult to ascertain when the first development of a given lesion was initiated.

The time required for the first signs of the disease was approximately arrived at by allowing the roots of tobacco and tomato plants to grow through the hole in the bottom of clay pots into water in small glazed crocks. When the protruding healthy white roots were sufficiently long, they were transferred to other crocks containing 1 to 2 inches of a heavy water suspension of brown root rot soil. At intervals individual plants were removed without appreciable disturbance of the individual roots and the soil was rinsed off the roots, which were again placed in pure water. In this manner it was found that approximately 3 days' exposure was required to secure the first

minute signs of the disease and that little or no further spread of the individual lesions occurred outside of the "infested" soil.

The relative concentration of the agent required to produce symptoms was determined by diluting the diseased soil with the same soil previously sterilized by heat (table 1). Depending upon the amount of brown root rot in the original soil, it required approximately 5 to 20 percent of infested soil to secure traces of brown root rot. At higher concentrations the amount of disease was roughly in direct proportion to the percentage of infested soil (fig. 2).

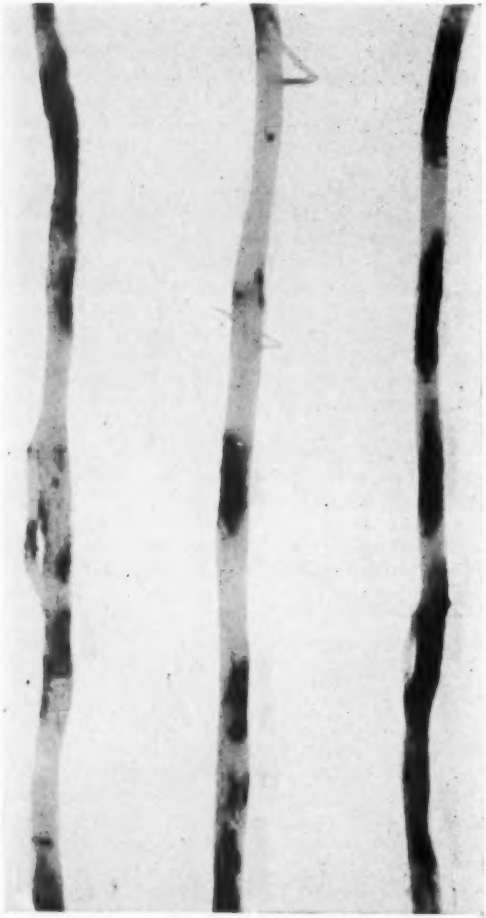


FIGURE 1.—Roots of tomato plants showing light-brown lesions normally characteristic of brown root rot. In severe cases of the disease the root system may entirely decay, with the result that the plants make little or no growth.

If diseased plants are transferred to disease-free soil they recover and new roots develop showing no further appreciable symptoms of brown root rot. Similarly, a change of weather conditions, which may either reduce the amount of the causal agent in the soil or check the rate of development of the disease (*11*) or both, often results in marked recovery in the same soil during the growing season. Young roots and young plants are most severely damaged by the disease. The lesions may be quite superficial on old roots and, as the plants grow older, new roots are likely to form faster than the disease progresses, with the result that recovery of plant growth may proceed rapidly once the balance has been shifted in favor of the host.

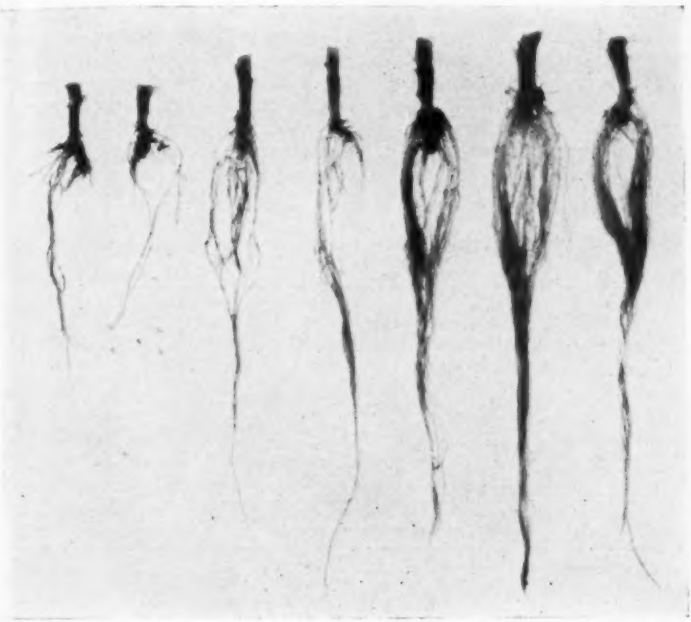


FIGURE 2.—Roots of tobacco plants showing comparative results in "infested" and disease-free soils and in mixtures in various proportions. If brown root rot soil is diluted with disease-free soil, the amount of disease in the roots is roughly in direct proportion to the percentage of the former soil in the mixture. From left to right, roots grown in 100, 80, 40, 20, 10, 5, and 0 percent of brown root rot soil.

TABLE 1.—*Influence of diluting 2 different brown root rot soils with sterilized soil on amount of disease developing upon roots of tobacco and tomato*

Brown root rot soil in mixture with sterilized soil (percent)	Amount [1] of brown root rot on—				Brown root rot soil in mixture with sterilized soil (percent)	Amount [1] of brown root rot on—			
	Tobacco plants grown in—		Tomato plants grown in—			Tobacco plants grown in—		Tomato plants grown in—	
	Soil 1	Soil 2	Soil 1	Soil 2		Soil 1	Soil 2	Soil 1	Soil 2
0	0	0	0	0	40	+++	+++	++	+
5	+	+	+	0	80	++++	++++	+++	++
10	++	+	+	0	100	++++	++++	+++	+++
20	++	++	+	+					

[1] 0=no disease; +=trace; ++=light; +++=moderate; ++++=heavy.

Previous results have shown that the brown root rot agent cannot be extracted from the soil by leaching with water and that it is apparently not present in even small amounts in filtered water extracts of the soil (*11*). These observations have been repeatedly corroborated. It has also been shown that fresh diseased roots will reproduce the disease when introduced into the soil (table 2). If such fresh diseased

roots are ground up and the juice is extracted, filtered through filter paper, and added to the soil, no disease is produced, although the pulp from which the extraction is made yields typical symptoms. Since the causal agent has not been secured in solution or suspension, it cannot be shown to be of any definite particle size. If the agent is of the nature of a toxin, it is possible that it might be dissolved by certain noninjurious chemicals, such as ether or acetone, and subsequently precipitated with other agents; but this has not yet been attempted.

TABLE 2.—*Effect of adding to disease-free soil brown root rot tomato roots treated in different ways*

Treatment of brown root rot roots added to soil	Amount of material added to 500 g of soil	Amount [1] of brown root rot on plants grown in—	
		Test 1	Test 2
Fresh roots.....grams..	18	++++	++++
Roots air-dried 24 hours.....do....	18	+	+
Roots sterilized.....do....	18	0	0
Fresh roots.....do....	25	++++	++++
Roots air-dried 24 hours.....do....	25	0	0
Extract from fresh roots.....cubic centimeters..	20	0	0
Extracted pulp from fresh roots.....grams..	10	++++	++++
Healthy fresh tomato roots (control).....do....	25	0	0

[1] 0=no disease; +=trace; ++++=heavy.

Since the agent is not soluble in water, further efforts were made to trace its distribution in the soil by means of physical fractionation of the soil with water. Brown root rot soil was shaken up repeatedly in large cylinders and allowed to settle out for definite periods of time. The soil used was very sandy so that about 95 percent settled out in 1 minute or less, and consequently considerable quantities had to be used to secure sufficient amounts of the silt and clay fractions for the test.

These preliminary trials showed that it was apparently impossible to wash the sand free from brown root rot with considerable quantities of water, since the sand fraction yielded quite as marked disease symptoms as the original soil. Approximately 1 part by weight of the soil fraction sedimenting between 1 and 24 hours from a 15-inch column mixed with 16 parts of sterile sand yielded nearly as much disease as did the control, showing that the agent was more concentrated in this silt portion than in the sand fraction. The finest clay or colloidal suspensions behaved like water extracts.

All the evidence goes to show that the brown root rot principle originates from or in the organic matter added to the soil through preceding crops; consequently, it is believed to be in highest concentration in such material or in its decomposition products. That organic matter carries the causal agent directly has been shown through the addition of the natural vegetable matter of brown root rot soils to disease-free soil, as well as by the use of diseased roots from brown root rot soil.

EFFECT OF DIFFERENT TEMPERATURES ON THE CAUSAL AGENT

Sterilizing brown root rot soil for a short period in the autoclave or in a steamer at 100° C. destroyed the disease-producing agent.

This behavior suggested that an organism capable of producing the disease was killed or that a toxic substance already present in the soil was destroyed. In seeking further information on this point, it was logical to determine more closely the actual temperature required to eliminate the causal agent.

A considerable quantity of soil was required to grow the necessary plants to determine the result of treatments, and consequently it was not possible to use short exposures to heat with a minimum quantity of soil. Beyond the commonly used 10-minute period of exposure for the thermal inactivation point, it was assumed that long exposures at low temperatures would yield more reliable results than short exposures, on account of the time required to heat soil to a uniform and definite temperature. With 1.2 kg. of moist soil spread out in a layer not more than 1 inch thick in a covered pan, the time of exposure in constant-temperature chambers was usually 24 to 48 hours. No appreciable drying of the soil took place under these conditions. The results presented in table 3 are sufficient to show that temperatures as low as 41° to 44° C., maintained for 24 hours, are very injurious to the brown root rot agent. At a temperature of 45° for 24 hours or 48° for 10 to 12 hours, the disease-producing agent is entirely eliminated. It was also found that a temperature of 45°, maintained for 24 hours, destroyed brown root rot in moist diseased roots subsequently used for inoculation in comparison with unheated diseased roots.

TABLE 3.—*Effect of heat on the causal agent of brown root rot, as determined by exposure of the soil to different temperatures for various periods*

Temperature (° C.)	Time of exposure	Amount [1] of brown root rot in—			
		Soil 1	Soil 2	Soil 3	Soil 4
	Hours				
48	48	0	0		
45	48			0	0
44	48	0	0		
41	48	0	0		
48	24	0	0		
45	24			0	0
44	24	+	0	0	+
43	24			+	+
41	24	++	++		
48	10–12			0	0
Unheated controls		++++	++++	++++	++++
100	⅓	0	0	0	0

[1] 0=no disease; +=trace; ++=light; ++++=heavy.

The maximum critical temperature for the growth of many microorganisms such as may be found in the soil is usually about 40° to 42° C., although most organisms are not killed by long exposures to temperatures of 45°. In experiments in which the same equipment was used as for the soil trials, *Radiobacter*, *Actinomyces scabies* (Thax.) Gues. and *Rhizoctonia bataticola* (Taub.) Butler all withstood 45° in culture for periods of more than 48 hours. These results appear to be especially significant in view of the report (*14*) that *R. bataticola* is associated with brown root rot. It is also worthy of note that the surface soil temperatures in the field may often reach 40° to 45° C. (104°–113° F.) for short periods during the day and that it is therefore

likely that summer heat accounts for the disappearance of some of the brown root rot disease from the soil under field conditions.

Experiments on the effect of freezing on brown root rot were made by placing 1.2-kg lots of soil in covered refrigerator pans and exposing these in refrigerator chambers at different constant temperatures as well as out of doors at the variable freezing temperatures during midwinter. The time of exposure to these conditions ranged from 4 to 60 days. It became obvious at once that temperatures sufficiently low were capable of destroying the brown root rot agent in the soil. The critical temperature and time of exposure have not been accurately determined. It must suffice to conclude that freezing at temperatures of −5° C. (23° F.) for as long as 21 days is not necessarily injurious to the causal agent but that temperatures of −25° C. (−13° F.) for 10 days may completely destroy brown root rot (table 4). Exposure to the variable winter temperature of Wisconsin for a period of about 1 month (mean, 20° F.) was also effective in eliminating the agent from soil exposed to the weather in covered containers. In one trial it was found that a temperature of approximately −5° C. for 8 days effectively destroyed the agent in diseased moist roots when these roots were used as soil inoculum in comparison with unfrozen roots from the same lot. Again, it is obvious that this reaction of the brown root rot agent to freezing is not in accord with what may be expected of a typical plant parasite. It is also apparent that the low prevailing temperatures of the winter months in the Northern States may destroy a large part of the brown root rot in the surface layers of soil, especially if the land is not protected by snow or other material.

TABLE 4.—*Effect of freezing on the causal agent of brown root rot, as determined by prolonged exposure of the soil and moist diseased roots to various low temperatures*

Treatment	Time of exposure	Amount [1] of brown root rot in—			
		Soil 1	Soil 2	Soil 3	Soil 4
	Days				
Soil exposed to—					
−5° C. (23° F.)	21	++++	++++		
−25° C. (−13° F.)	26	0	0		
Control (soil not frozen)		++++	++++		
Soil exposed to—					
−25° C	33			0	0
−25° C	10			0	0
−25° C	4			+	++
Outdoor temperature (20° F., mean)	33			0	0
Control (soil not frozen)				++++	++++
Moist diseased roots [2] exposed to −5° C	8			0	0
Control (moist diseased roots)				++++	++++

[1] 0=no disease; +=trace; ++=light; ++++=heavy.
[2] Frozen before being added to sterilized soil.

EFFECT OF DRYING ON THE CAUSAL AGENT

It was shown in earlier studies on brown root rot (*11*) that air-drying the soil effectively eliminates the disease. This result has since been repeatedly corroborated. The method of drying used in the first experiments consisted simply in spreading the soil out in a thin layer in a dry room or out of doors on a clear day. In more recent tests the rate of drying has been more carefully regulated under controlled environmental conditions or in a partly covered container.

The data secured show quite conclusively that quick air-drying in as short a period as 5 hours is quite as effective as much slower drying. Very gradual air-drying of the soil over a period of several days or weeks, on the other hand, is far less destructive of brown root rot

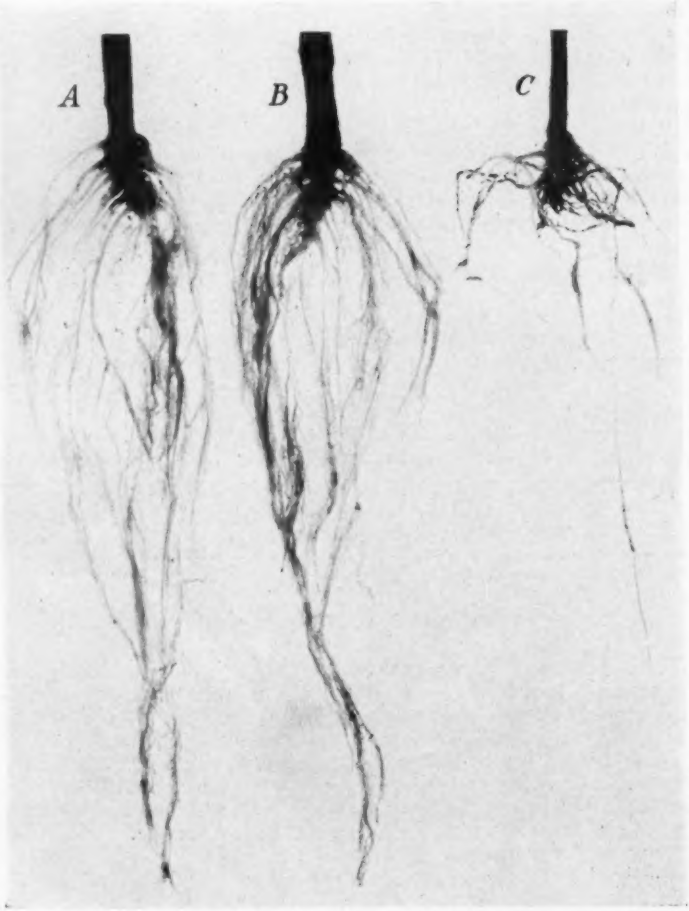


FIGURE 3.—Roots of tomato plants grown in soil to which roots affected with brown root rot have been added: *A*, Roots from soil containing sterilized diseased roots; *B*, roots from soil containing dried diseased roots; *C*, roots from soil containing fresh diseased roots. *A* and *B* failed to develop disease, whereas *C* became diseased.

(table 5). In one instance, soil was maintained in a weighed covered pan for 120 days at 32° C. After 90 days, the soil lost no further weight, being completely air-dry. This slowly dried soil, when planted to tomatoes, yielded comparatively good symptoms of brown

root rot. No reliable explanation can be offered at present for this behavior.

Little is known about possible related phenomena, but the similarity of this behavior to the inactivation of the virus of ordinary tobacco mosaic in the soil by desiccation (*8*) is interesting and suggests that the disease-producing agent of brown root rot may be adsorbed by the drying soil. This explanation is hardly tenable, however, since desiccation is effective in destroying the active principle of brown root rot in the plant tissues as well (fig. 3), which is not true of the mosaic virus.

It is believed that the drying of the soil and possibly the rate at which drying takes place are of considerable significance in relation to the epidemiology of the disease. In the absence of a more definite means of diagnosis, air-drying may serve as a valuable method of identifying the typical brown root rot in doubtful cases.

TABLE 5.—*Effect on the causal agent of brown root rot of drying soil at different rates at 32° C.*

[Rate of drying regulated by covers on pans]

Time of drying (days)	Total loss of water from—		Amount [1] of brown root rot in—		Time of drying (days)	Total loss of water from—		Amount [1] of brown root rot in—	
	Soil 1	Soil 2	Soil 1	Soil 2		Soil 1	Soil 2	Soil 1	Soil 2
	Percent	*Percent*				*Percent*	*Percent*		
2	15.0	12.4	+	0	28	14.8	12.3	++++	+++
8	12.7	10.8	++	++	None (control)	0	0	++++	++++

[1] 0=no disease; +=trace; ++=light; +++=moderate; ++++=heavy.

EFFECT OF AERATION AND LIGHT ON THE CAUSAL AGENT

The exposure of brown root rot soil to drying by ordinary means also exposes the soil to greater aeration, a factor that may conceivably influence the causal agent through oxidation. In the earlier experiments, some attempts were made to aerate the soil for several days, without drying, by spreading it in a thin layer in a moist atmosphere (*11*). A later modification of this method consisted of the use of covered and open containers, with daily stirring for several weeks, and the maintenance of moisture content daily by weight. Results from this type of experiment are shown in table 6. Aeration by these methods had little or no effect on the amount of brown root rot.

Complete drying of considerable quantities of soil without aeration, or thorough aeration without drying at room temperatures, is not easily accomplished even with special equipment developed for the purpose. The first equipment used consisted of a slowly rotating glass cylinder (4 by 18 inches) for continuous stirring, through which was constantly passed an atmosphere regulated as to moisture and oxygen content (fig. 4). Some difficulties were encountered because of moist soil adhering to the walls of the revolving glass cylinder, but again it was found that the presence of pure oxygen for a week or its complete absence in continuously agitated soil had no effect on the

brown root rot disease. More satisfactory results with less mechanical difficulties were later secured by the use of large side-tube desiccators to permit the change of atmosphere at intervals only. The

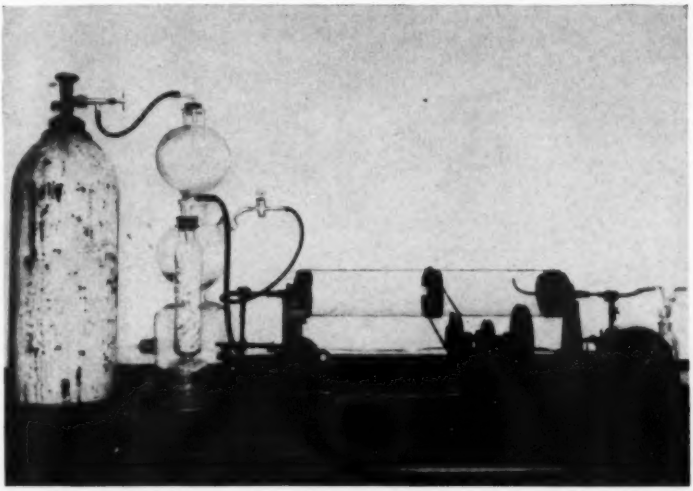


FIGURE 4.—Special equipment used for aerating brown root rot soil without drying, or vice versa. The soil is exposed in the slowly rotating glass cylinder for 7 or more days to atmosphere regulated as desired in oxygen and moisture content.

drying agent used was calcium chloride. Nitrogen, oxygen, and air were introduced and replaced as desired. Other modifications per-

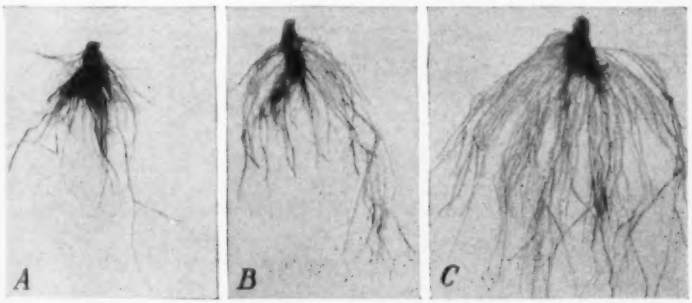


FIGURE 5.—Roots of tobacco plants grown in brown root rot soil: *A*, In moist unaerated soil; *B*, in moist aerated soil; *C*, in soil dried during the period of aeration. Aeration of moist brown root rot soil does not appreciably reduce the amount of disease.

mitted occasional stirring of the soil without disturbing the existing atmosphere in the container. In this manner, about 400 gm. of moist soil could usually be dried in 7 days in the presence or absence of air or pure oxygen.

The results of many trials, some of which are shown in table 7, further demonstrate quite conclusively that aeration (fig. 5) or pure oxygen as such has comparatively little if any influence on the persistence of brown root rot in the soil. The effect of drying on brown root rot soil, therefore, cannot be attributed even in small part to direct oxidation of an injurious chemical substance that may be the responsible agent.

TABLE 6.—*Effect of drying as compared with aeration on the causal agent of brown root rot*

[2,000 g of soil in refrigerator pans treated as shown for 30 days]

Treatment of soil	Moisture		Amount [1] of brown root rot after treatments
	Average daily loss	Average daily supply	
	Grams	*Grams*	
Not stirred (pan covered)	1	1	++++
Stirred daily (pan covered)	1	1	++++
Stirred daily (pan open)	40—0	0	0
Do	40	40	++++

[1] 0=no disease; ++++=heavy.

TABLE 7.—*Effect on the causal agent of brown root rot of exposing soil to oxygen, nitrogen, and air for 7 days, with and without drying*

[Atmosphere regulated in closed container and soil stirred frequently]

Atmosphere	Soil condition at end of exposure	Amount [1] of brown root rot after treatment		Atmosphere	Soil condition at end of exposure	Amount [1] of brown root rot after treatment	
		Soil 1	Soil 2			Soil 1	Soil 2
Oxygen	Moist	+++	+++	Nitrogen	Dry	0	+
Nitrogen	do	+++	+++	Air	do	+	+
Air	do	+++	+++	Control (no treatment)		+++	+++
Oxygen	Dry	0	+				

[1] 0=no disease; +=trace; +++=moderate.

Some farmers believe it is possible to control the disease in the field by aerating the soil through several deep cultivations prior to planting. Any benefits secured from such cultivation probably result from increased exposure of the soil to drying, a condition which could hardly be expected to be, and in practice evidently is not, effective in seasons of normal rainfall.

It is also conceivable that the exposure of brown root rot soil to sunlight may reduce the disease during drying or aeration experiments, especially where the soil is continually or repeatedly stirred or agitated in the presence of strong sunlight. To test this possibility, brown root rot soils were exposed to drying and to aeration without drying, both in the presence and in the absence of light. No reduction in brown root rot that could be attributed to light was found in these trials (table 8).

TABLE 8.—*Effect of light as compared with drying on the causal agent of brown root rot in soil*

Sunlight	Time of exposure	Soil condition at end of exposure	Amount [1] of brown root rot in—		Sunlight	Time of exposure	Soil condition at end of exposure	Amount [1] of brown root rot in—	
			Soil 1	Soil 2				Soil 1	Soil 2
	Hours					*Hours*			
Subdued	24	Moist	+++	+++	Strong	5	Moist	+++	+++
Do	24	Dry	0	0	Do	5	Dry	0	0
None	24	do	0	0	Control		Moist	+++	+++

[1] 0=no disease; +++=moderate.

EFFECT OF CHEMICALS ON THE CAUSAL AGENT

The high susceptibility of the causal agent of brown root rot to certain physical conditions suggests that it also may be intolerant to low concentrations of various chemicals added to the soil. It was especially hoped that some correlation might be found between the reaction of brown root rot to certain chemicals and the germicidal properties of such chemicals. In this respect the results are somewhat conflicting, but wholly satisfactory data are not easily secured in some instances since the concentration of nonvolatile chemicals used must of necessity be below that seriously toxic to the host or test plant used. Silver nitrate, copper sulfate, boric acid, and lithium carbonate, for example, were found to be extremely toxic in low concentrations in the soil. Although brown root rot was not entirely eliminated by the concentrations of these chemicals that could be used, it is likely that somewhat higher concentrations might have been more destructive to brown root rot. The destructive chemicals shown in table 9, though widely different as regards germicidal properties, completely eliminated brown root rot from the soil. Of these, only formalin and acetic acid (*3*) may be regarded as effective soil fungicides of known value, although all the others, with the possible exception of charcoal, possess more or less germicidal properties. The behavior of charcoal is especially interesting and again suggests adsorption of the causal agent as a possible explanation. In the gaseous form, chloroform, toluene, xylol, ammonia, formalin, carbon bisulfide, and ethylene chlorhydrin were all very effective as destructive agents.

Quite as significant is the list of chemicals that failed to affect brown root rot appreciably. Substances that alter the reaction of the soil to a considerable degree (hydrochloric acid, sodium hydroxide, calcium carbonate, and sulfur) may have been expected to give some degree of modification in brown root rot, but this was not apparent although the soil reaction was varied from pH 4.6 to 7.7. Potassium permanganate produced interesting chemical "lesions" on the roots, which, at low concentrations of the chemical, resembled those of brown root rot. The disease lesions were nevertheless also conspicuously present. Other chemicals, e. g., boric acid, may be very toxic to the foliage and stems of tomato without showing marked toxicity to the roots or to brown root rot. The failure of naphthalene vapor and Cynogas to affect the disease offers some presumptive evidence that neither nematodes nor other soil fauna are concerned. The differential behavior of chloroform and ether seemed at first rather significant.

However, when cultures of different organisms were exposed to these chemicals under similar conditions, it was apparent that chloroform inhibited fungi and bacteria much more completely than did ether.

TABLE 9.—*Chemical substances tested on brown root rot at concentrations* [1] *permitting the subsequent growth of tomato plants in the treated soil*

Chemicals destroying causal agent			Chemicals not destroying causal agent		
Chemical	Concentration or physical state	Amount used on 1.2 kg of soil	Chemical	Concentration or physical state	Amount used on 1.2 kg of soil
Alcohol (ethyl)	50 percent	200 cc	Aluminum hydroxide (dry)		24 g
Ammonium hydroxide (c. p.)	Gas	10 cc	Ammonium sulfate	1 percent	200 cc
Acetic acid	1 percent	200 cc	Boric acid	0.5 percent	200 cc
Carbon bisulfide	Fumes	10 cc	Calcium carbonate (dry)		48 g
Charcoal (dry)		24 g	Copper sulfate	0.1 percent	200 cc
Chloroform	Fumes	10 cc	Cynogas (commercial dry)		10 g
Ethylene chlorhydrin	10 percent	200 cc	Ether	Fumes	20 cc
Formaldehyde (40 percent)	Gas	10 cc	Hydrogen peroxide	3 percent	200 cc
Mercuric chloride	0.2 percent	200 cc	Hydrochloric acid	1.5 percent	200 cc
Toluene (c. p.)	Gas	10 cc	Infusorial earth (dry)		24 g
Xylol (c. p.)	do	10 cc	Kaolin (dry)		24 g
			Lithium carbonate	0.2 percent	200 cc
			Naphthalene (dry)	Gas	10 g
			Potassium permanganate	0.5 percent	200 cc
			Sodium hydroxide	0.2 percent	200 cc
			Sodium nitrate	2 percent	200 cc
			Silver nitrate	0.1 percent	200 cc
			Sulfur (dry powder)		4 g
			Talc (dry powder)		24 g
			Zinc sulfate	0.1 percent	200 cc

[1] The treatments with gases and fumes were made in a closed 5-liter container in which the amount of chemical shown was evaporated from a shallow dish.

Although the preliminary results of the experiments with chemicals do not in themselves offer any direct explanation of the nature of brown root rot, they are of some special interest in connection with further means of identification of the causal agent and suggest also possibilities of a practical control of the disease through chemical sterilization of the soils. Further studies should be made of the influence on the normal flora and fauna of the soil of chemicals that inhibit brown root rot. Such investigations should permit the elimination of certain species or groups of micro-organisms as probable agents in the production of the disease.

SUSCEPTIBILITY OF PLANT SPECIES TO THE DISEASE

In the earlier trials reported from this laboratory (*11*), the following plants were named as attacked by brown root rot: Tobacco, tomato, potato, eggplant, pepper, cowpeas, soybeans, garden beans, hairy vetch, and clovers. A considerable variation in relative susceptibility of the plants was noted at the time. Plants found difficult to classify included corn, oats, barley, wheat, rye, and timothy. Species apparently immune included cabbage, onion, beet, pumpkin, cucumber, and lettuce.

In recent experiments, most of these plants and a considerable number of other species have again been grown in brown root rot soil, and their relative susceptibility to the disease has been approximately determined. Such plants are listed in table 10 as showing three degrees of susceptibility; also included in table 10 is a list of plants that are apparently immune according to these tests. The plants tested were selected chiefly with a view to including the more common

crop and ornamental plants, a small group of weeds, and another group of the Leguminosae. The legume family was selected for special consideration largely because of the suspected susceptibility of its species to the disease and their common use as soil-improving crops. Despite the number of very susceptible species in this family, preliminary results indicate that there may be some variation in susceptibility between different varieties of the same species, especially in the varieties of *Pisum sativum* L., but further trials are needed to establish this point.

TABLE 10.—*Plant species showing approximate relative susceptibility to brown root rot*

Very susceptible	Susceptible	Resistant	Immune
Tobacco (*Nicotiana tabacum* L.). Tomato (*Lycopersicon esculentum* Mill.). Cotton (*Gossypium hirsutum* L.). Okra (*Hibiscus esculentus* L.). Snapdragon (*Antirrhinum majus* L.). Sunflower (*Helianthus annuus* L.). Cornflower (*Centaurea cyanus* L.). Bushy arctotis (*Arctotis stoechadifolia* Berg.). Leguminosae: Cowpea (*Vigna sinensis* (Torner) Savi). Soybean (*Soja max* (L.) Piper). Common bean (*Phaseolus vulgaris* L.). Common pea (*Pisum sativum* L.). Peanut (*Arachis hypogaea* L.). Locust (*Robinia pseudoacacia* L.). Sesbania (*Sesbania punicea* (Cav.) Benth.). Fenigreek (*Trigonella foenum-graecum* L.). Tepary bean (*Phaseolus acutifolius latifolius* Freem. Civet bean (*Phaseolus lunatus* L.). Velvetbean (*Stizolobium deeringianum* Bort.). Hairy vetch (*Vicia villosa* Roth). Vetch (*Vicia grandiflora* Scop., *V. atropurpurea* Desf., *V. pannonica* Crantz).	Potato (*Solanum tuberosum* L.). Eggplant (*Solanum melongena* L.). Pepper (*Capsicum annuum* L.). Nightshade (*Solanum miniatum* Bernh.). China-aster (*Callistephus chinensis* (L.) Nees). Lettuce (*Lactuca sativa* L.). Nasturtium (*Tropaeolum majus* L.). Common basil (*Ocimum basilicum* L.). *Gaillardia* sp. California-poppy (*Eschscholtzia california* Cham.). Marjoram (*Majorana hortensis* Moench). Spurge (*Euphorbia* sp.). Leguminosae: Lentil (*Lens esculenta* Moench). Alsike clover (*Trifolium hybridum* L.). Alfalfa (*Medicago sativa* L.). Lespedeza (*Lespedeza striata* (Thunb.) Hook. and Arn.). Mung bean (*Phaseolus aureus* Roxb.). Pigeonpea (*Cajanus indicus* Spreng.). Partridge-pea (*Chamaecrista fasciculata* (Michx.) Greene). (*Desmanthus illinoensis* (Michx.) MacMil.). Trefoil (*Lotus corniculatus* L.).	Groundcherry (*Physalis pubescens* L.). Apple-of-Peru (*Nicandra physaloides* (L.) Pers.). Buckwheat (*Fagopyrum esculentum* Moench). Hemp (*Cannabis sativa* L.). Common beet (*Beta vulgaris* L.). Spinach (*Spinacia oleracea* L.). Wheat (*Triticum vestivum* L.). Timothy (*Phleum pratense* L.). Broomcorn millet (*Panicum miliaceum* L.). Rice (*Oryza sativa* L.). Sudan grass (*Sorghum vulgare sudanense* (Piper) Hitchc.). Quackgrass (*Agropyrum repens* (L.) Beauv.). Calendula (*Calendula officinalis* L.). Garden balsam (*Impatiens balsamina* L.). Catchfly (*Silene noctiflora* L.). Pigweed (*Amaranthus retroflexus* L.). Sheep sorrel (*Rumex acetosella* L.). Wallflower (*Cheiranthus cheiri* L.). Fennel (*Foeniculum vulgare* Hill). Dill (*Anethum graveolens* L.). Nemesia (*Nemesia strumosa* Benth.). Gilliflower (*Matthiola* sp.). Leguminosae: Sweet pea (*Lathyrus odoratus* L.). White clover (*Trifolium repens* L.). Milkvetch (*Astragalus* sp.). (*Lathyrus hirsutus* L.). Beggarweed (*Desmodium tortuosum* (Sw.) DC. Crownvetch (*Coronilla varia* L.). Spring vetch (*Vicia sativa* L.). Vetch (*Vicia picta* Fisch. and Mey.).	Jimsonweed (*Datura stramonium* L.). Onion (*Allium cepa* L.). Pumpkin (*Curcurbita pepo* L.). Cucumber (*Cucumis sativus* L.). Cabbage (*Brassica oleracea capitata* L.). White mustard (*Brassica alba* (L.) Boiss.). Barley (*Hordeum vulgare* L.). Oats (*Avena sotiva* L.). Rye (*Secale cereale* L.). Corn (*Zea mays* L.). Bluegrass (*Poa bulbosa* L.). Reed canary grass (*Phalaris arundinacea* L.). Chicory (*Cichorium intybus* L.). Pokeweed (*Phytolacca decandra* L.). Plantain (*Plantago major* L.). Bindweed (*Convolvulus sepium* L.). French weed (*Thlaspi arvense* L.). Campion (*Lychnis alba* Mill.). Sweet-william (*Dianthus barbatus* L.). Coneflower (*Rudbeckia laciniata* L.). Stock (*Matthiola incana* (L.) R. Br.). Drummond phlox (*Phlox drummondii* Hook.). Scabiosa (*Scabiosa atropurpurea* L.). Butterflyflower (*Schizanthus* sp.). Milkweed (*Asclepias syriaca* L.). Zinnia (*Zinnia elegans* Jacq.).

The comparative influence of plants immune and susceptible to brown root rot, when used as preceding crops for tobacco, is not yet definitely known. It is only known that the use of certain susceptible plants like tobacco in "diseased" soil neither maintains nor increases the amount of disease in subsequent years, but rather strikingly decreases the disease. This circumstance may, as has been shown, be due in considerable measure to the greater exposure of cultivated soils to the influence of the weather.

The increased host range presented, it is hoped, may aid in the selection of plants for further experimental work. Some of the new hosts should prove especially suitable for new etiological studies.

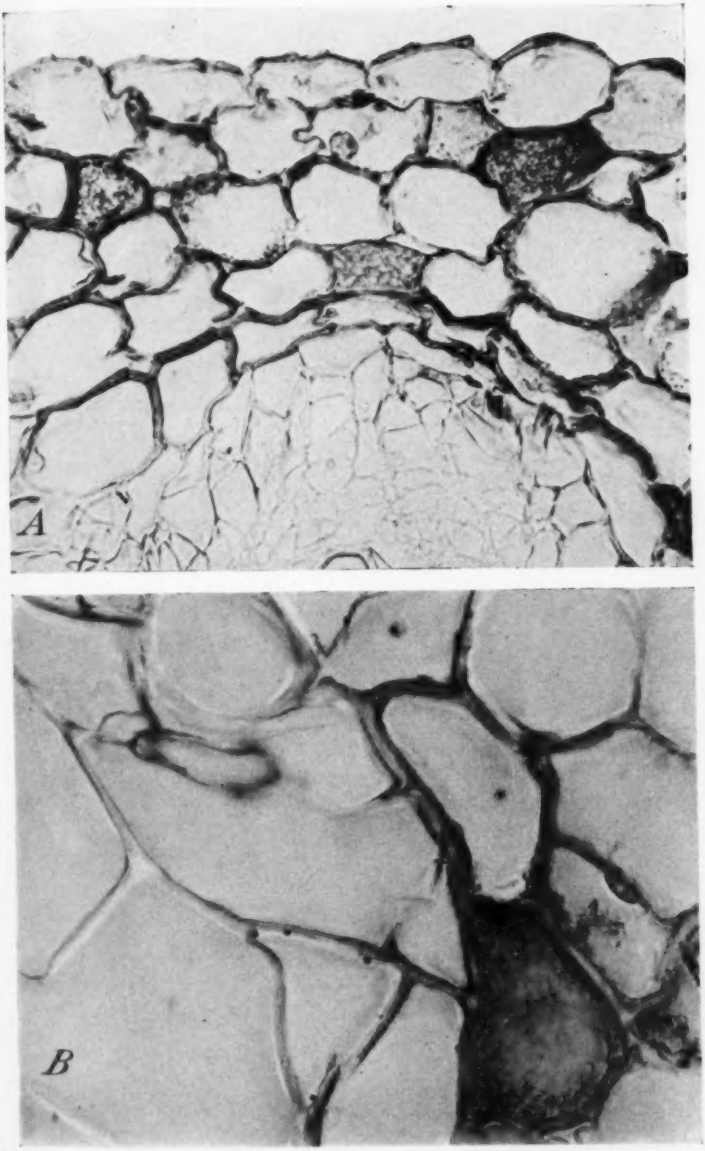
PATHOLOGICAL HISTOLOGY OF THE DISEASE[4]

Earlier microscopic examination of the exterior of diseased roots and lesions torn apart with needles frequently showed the presence of abundant hyphae of fungi that appeared to be closely associated with the disease. A form of *Rhizoctonia* that was especially common (*11*) was isolated with some difficulty but proved not to be capable of reproducing the disease.

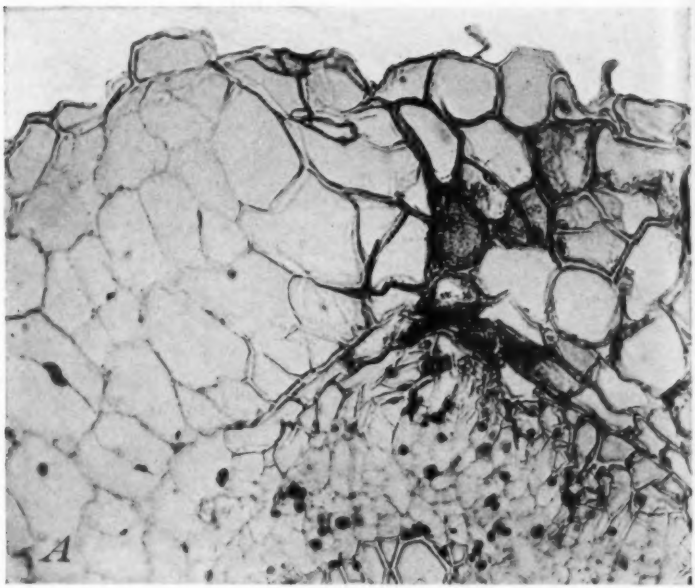
Examination of the diseased tissue by means of freehand and paraffin-sections failed to show either frequent or extensive invasion of the affected cells by fungi of any sort. The yellowing of the cell walls and the early granulation of the cell contents followed by decay of the cells in the late stages of the disease were the only evident histological features present. The failure of other means of study to yield conclusive results on the nature of the disease seemed, however, to warrant a more comprehensive reexamination of the affected tissues by histological methods. This work was undertaken and conducted chiefly with greenhouse-grown material from various soils collected in 1934 and 1935 from Massachusetts, Wisconsin, and Maryland fields, although some further study with improved techniques was made from paraffined material fixed prior to 1926 and as early as 1918. Over 150 separate specimens of paraffined root tissue were cut, stained, and mounted, involving the examination of several thousand sections. In addition, sections were cut from about 60 specimens of roots that had been previously wounded mechanically (by needle punctures, razor cuts, etc.), injured by chemical agents (phenol, ammonium carbonate, etc.), or infected with black root rot (*Thielaviopsis basicola*) for comparison with brown root rot lesions.

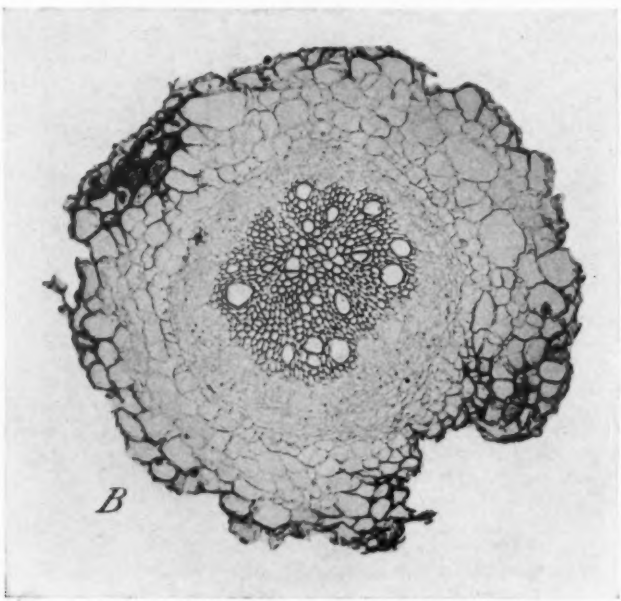
Practically all of the histological studies were made on tobacco and tomato roots. Vigorous roots from young plants, with lesions of varying ages, were usually selected. Out of 10 fixatives tried, Karpechenko's gave the best results. Specimens about one-fourth of an inch in length were fixed, washed, and dehydrated in butyl or ethyl alcohol, cleared in cedarwood oil, and embedded in paraffin at 51° to 52° C. Sections were cut 8μ or 10μ thick; staining methods used included 16 single, 5 double, and 2 triple stains. Best results were secured with cyanine and erythrosin prepared according to Chamberlain's general formula for aniline dyes. Mallory's triple stain, methyl green, and Congo red also gave fair results. Typical brown root rot gives early a distinctly yellow or brown coloration to

[4] The writer is indebted to Elmer McMurry for able assistance in carrying out this phase of the investigation.



Sections of tobacco root affected with brown root rot. *A*, Early stage of the disease, in which sharply delimited cells filled with finely or coarsely granulated material are conspicuous. Neither in these nor in neighboring cells is there any evidence of a micro-organism regularly associated with the disease. In *B* the contrast between healthy and diseased areas is shown, the cell walls in the latter being yellowed and thickened. *A*, × 500; *B*, × 1,200.





A, Section of tobacco root affected with brown root rot. The disease frequently penetrates the cortex to the endodermal region, where the spread may be more rapid in a lengthwise direction. *B*, Cross section of tobacco root affected with brown root rot, showing injury to epidermal cell layers around entire root, with deep lesions in two areas. *A*, × 500; *B*, × 180.

the cell wall and cell contents, so that it is often desirable to study the sections without staining.

One of the most striking characteristics of brown root rot lesions is the sharp demarcation by the cell walls of the diseased areas from healthy surrounding tissues. In the early stages of the disease or at the margins of the lesions it is not uncommon to find stray single diseased cells in the epidermal layer or deep in the cortical tissue. Examination of the younger lesions under high powers shows the entire cell protoplasm to be disintegrated, only finely or coarsely granular material being present (pl. 1, *A*). Neither in these affected cells nor in cells surrounding them is it possible to find the regular presence of any foreign organism. Occasional fungus strands and nematodes may be seen, but because of their irregular association with the disease, they are evidently secondary invaders. The granular structure of cell material is not suggestive of bacteria nor does it appear that bacteria may be associated with its formation. On the basis of extensive studies of paraffin sections, it appears most unlikely that internal parasitic organisms are concerned with the malady. The possibility of micro-organisms on the root surfaces affecting the root tissues deleteriously through secretions is not obviated by these histological methods.

An outstanding feature of the affected tissue is the thickening of the cell walls (pl. 1, *B*). It is likely that the cell wall is the first to show signs of disease through yellowing, followed by gradual thickening to two or three times its normal width. It has not been possible to observe earlier stages of the disease, either because of the low content of cytoplasm in the root areas used or because the staining has not been suitable for the purpose. The nuclei evidently disintegrate in diseased cells, as shown by their modified reaction to stains, but further intermediate stages of the nuclei or of the cytoplasm that might account for the cell granulations have not been observed.

With this picture of the pathological condition of the cells, it is possible to follow the progress of the disease in the roots to a considerable extent. It is clear from gross examination that a large number of local lesions are normally responsible for extensive root injuries and that brown root rot starts and progresses very slowly. The disease is often plainly initiated at the point of emergence of secondary rootlets, but no evidence has been found that would indicate any limitation to this area since single epidermal cells or small groups of cells elsewhere are not infrequently found to be diseased. From such initial epidermal flecks the lesion may spread in all directions. If the spread is largely superficial, it may involve the periphery to a depth of only one or two cells for some distance. More commonly the lesion spreads laterally through the cortex until the endodermal region is reached (pl. 2, *A*). Here the resistance to the progress of the disease seems to be less than elsewhere, and the stele is soon surrounded; then follows a more rapid longitudinal spread of the disease between the stele and the cortex. This often results in the separation of the cortex from the stele and accounts for the fairly common macroscopic symptom of sloughing off of the living cortex from diseased roots, leaving only the threadlike central cylinder in young roots. In young roots also, before the interfascic-

ular phloem has been developed, the disease occasionally penetrates to the xylem by means of a medullary ray. After secondary phloem encircles the stele, infection rarely penetrates—the phloem acting as a barrier—although occasional pericycle cells and outer secondary phloem are distinctly affected for some distance.

In older roots, the disease is less likely to reach the endodermal region and the lesions may be limited entirely to the cortical layer, causing deep gashes and scars in the roots, which nevertheless may never completely succumb to the disease (pl. 2, *B*). Some evidence of the development of a physical barrier to the progress of the disease has been frequently observed. The development of meristematic tissues in pericycle or cambial layers below the lesion appears to aid in protecting the area of the stele from disease invasion in somewhat the manner described by Conant (*2*) for black root rot. Whatever the normal or stimulated barrier may be, as secondary thickening develops in the roots they become less predisposed to brown root rot and less injury results from developing lesions.

The microscopic examination of brown root rot specimens from the several different soils of the Connecticut Valley, Maryland, and Wisconsin showed very similar pathological histology and suggests the causal agent to be the same in all instances.

It is of some special interest and value in the present connection to compare the pathological histology of brown root rot with lesions produced by more definite causal agents, such as toxic chemicals, mechanical wounds, or parasitic fungi. The treatments were made on free roots grown from the base of potted plants suspended over crocks containing water or the chemical solution used. The roots could therefore be examined at any time for purposes of selecting specimens for fixation. Of many chemicals tested, none was found that would produce external symptoms similar to those of brown root rot, although some at the proper strength produced comparable symptoms. The mechanical injuries on the roots were made by needle punctures, razor cuts, and pinching with tweezers. The parasitic lesions were produced by *Thielaviopsis basicola* on tobacco. Without describing in detail the obvious and characteristic macroscopic differences between lesions produced by the methods described and lesions found in brown root rot, it is worthy of note that close replicas of the histological picture of brown root rot could be found in all types of cell injury, namely, cell-wall discoloration, cell-wall thickening, and granulation of cell contents. In general, the fundamental features of the pathological histology of brown root rot do not appear to be specific for the disease, and while they do not exclude the possibility that a toxin of some sort is involved, the failure to find a microscopically visible organism in or between the diseased cells lends support to the idea that a typical parasite is not involved.

DISCUSSION

The results presented in this paper are chiefly of interest in relation to the nature of the causal agent of brown root rot. Evidence for and against a parasitic cause of the disease was presented earlier (*11*), and both hypotheses have received some support from later workers (*4*, *14*). Some phases of the problem remained clearly in need of more detailed study, namely, the behavior and properties of the causal

agent and the pathological anatomy or histology of the disease. The studies on properties have shown to a more striking degree than previously the extreme sensitivity of the causal agent to certain environmental conditions. Although the elimination of the causal agent from the soil by sterilizing at 100° C., for example, is suggestive, its destruction at 45° is of much greater significance as bearing on its nature. This and other properties of the causal agent should aid materially in eliminating many organisms suspected of having a causal relation to the disease and should give a more specific idea as to what type of organism or substance may be concerned.

Similarly, the histological picture of the disease, and more particularly the failure to find any evidence of a constantly associated organism in the diseased tissues in a wide variety of material, may be regarded as highly contradictory evidence against a parasitic agent of the usual nature. It does not disprove the possibility that organisms confined largely to the exterior of the roots or harbored only in the soil itself may excrete toxic substances that are necrotically injurious to the roots. That fungi produce toxins has long been known and recently has been studied, perhaps most interestingly by Weindling (*18*). Such an assumption perhaps fits most closely the theory suggested by Thomas (*17*).

Although several investigators have reported that decomposing vegetable matter in or out of the soil produces substances toxic to plant growth and to plant roots, much remains to be explained before this hypothesis can be broadly accepted to explain brown root rot. If this explanation should prove to be true it is likely that a specific organism constantly associated with brown root rot soils dominates the decomposition, forming a product that will produce typical brown root rot symptoms. Such an organism or the toxin it produces will probably show the high sensitivity indicated by the behavior and properties of the causal agent of brown root rot. Some evidence has been secured that certain pure cultures of fungi (*Aspergillus terreus* Thom) will produce materials toxic to roots under certain conditions of nutrition, but thus far no typical brown root rot has been secured. Further work of this general type is one of the few remaining obvious methods of attacking the brown root rot problems.

SUMMARY

The true nature of the causal agent of brown root rot is not known. The present paper is concerned chiefly with the behavior and properties of the causal agent and the pathological histology of the affected tissues.

The causal agent is relatively slow in its action on roots and is not highly concentrated in the soil as judged by dilution experiments. The active principle is very sensitive to certain physical conditions and to some chemical substances. It is intolerant to rapid air-drying of the soil but may withstand prolonged slow drying. It is destroyed by heat at a temperature as low as 45° C. in a period of 24 hours and by moderate freezing temperatures maintained for a relatively long period. Aeration and light do not appear to have any injurious effect. Brown root rot is eliminated from the soil by several chemicals that have germicidal properties, as well as by powdered charcoal,

151679—39——6

whereas other chemical substances with similar properties have little or no effect.

Tobacco and a wide variety of other plants are susceptible to brown root rot. The legume family is particularly susceptible. Susceptibility to the disease is apparently not correlated with the origin or persistence of the causal agent in the soil. The reduction of the causal agent in the soil is apparently dependent in large part on the exposure of cultivated soils in particular to extremes of heat, cold, and drought.

The histological and cytological examination of diseased tissues failed to show any organism to be constantly associated with the disease. The microscopic features of the disease are chiefly the yellowed and thickened cell walls, discolored and granulated cell contents, and sharply delimited diseased and normal cells. The lesions may often be superficial but generally extend through the cortex of young roots, after which a more rapid and extensive spread may develop around the stele.

The results of the present investigation offer no direct proof of the nature of the causal agent of brown root rot but should aid materially in the conduct of further studies on the etiology of the disease.

LITERATURE CITED

(1) Beaumont, Albert B.
1936. A hypothesis to explain brown root rot of Havana seed tobacco. Science 84: 182–183.

(2) Conant, George H.
1927. Histological studies of resistance in tobacco to Thielavia basicola. Amer. Jour. Bot. 14: 457–480, illus.

(3) Doran, William L.
1928. Acetic acid as a soil disinfectant. Jour. Agr. Research 36: 269–280, illus.

(4) ———
1928. The growth of tobacco and brown root rot of tobacco as affected by timothy infusions of different ages. Jour. Agr. Research 36: 281–287.

(5) Eisenmenger, Walter S.
1934. The forms of nitrogen in infusions of corn, timothy, red clover, tobacco, and red top. Jour. Agr. Research 49: 375–378.

(6) ———
1938. Some correlations in plant-tissue composition, decomposition products, and effect upon crop rotation with tobacco. Jour. Agr. Research 56: 309–316.

(7) Garner, W. W., Lunn, W. M., and Brown, D. E.
1925. Effects of crops on the yields of succeeding crops in the rotation, with special reference to tobacco. Jour. Agr. Research 30: 1095–1132, illus.

(8) Hoggan, Ismé A., and Johnson, James.
1936. Behavior of the ordinary tobacco mosaic virus in the soil. Jour. Agr. Research 52: 271–294.

(9) Johnson, James.
1938. Brown root rot of tobacco. (Abstract) Phytopathology 28: 11.

(10) ——— and Ogden, W. B.
1930. Rotation and the tobacco crop. Wis. Agr. Expt. Sta. Bull. 412, 24 pp., illus.

(11) ——— Slagg, C. M., and Murwin, H. F.
1926. The brown root rot of tobacco and other plants. U. S. Dept. Agr. Bull. 1410, 30 pp., illus.

(12) Jones, J. P.
1929. The effect of other crops on tobacco. Jour. Amer. Soc. Agron. 21: 118–129.

(13) LOEHWING, WALTER F.
1937. ROOT INTERACTIONS. Bot. Rev. 3: 195–239.
(14) MATHEWS, EARLE D., RENEGER, C. A., and THOMAS, R. P.
1934. MANAGEMENT OF SOILS SUBJECT TO TOBACCO BROWN ROOT ROT. Md. Agr. Expt. Sta. Bull. 362: 393–394.
(15) MURWIN, H. F.
1929. BROWN ROOT ROT. Mass. Agr. Expt. Sta. (Bien. Rept.) Bull. 247: 341–342.
(16) SWANBACK, T. R., and JACOBSON, H. G. M.
1933. BROWN ROOT ROT OF TOBACCO. Science 77: 169.
(17) THOMAS, R. P.
1930. THE RELATION OF NITRATE NITROGEN AND NITRIFICATION TO THE GROWTH OF TOBACCO FOLLOWING TIMOTHY. Wis. Agr. Expt. Sta. Research Bull. 105, 28 pp., illus.
(18) WEINDLING, R.
1934. STUDIES ON A LETHAL PRINCIPLE EFFECTIVE IN THE PARASITIC ACTION OF TRICODERMA LIGNORUM ON RHIZOCTONIA SOLANI AND OTHER SOIL FUNGI. Phytopathology 24: 1153–1179, illus.

RELATION OF THE STRUCTURE OF THE CHALAZAL PORTION OF THE COTTON SEED COAT TO RUPTURE DURING GINNING [1]

By NORMA L. PEARSON [2]

Associate cotton technologist, Bureau of Plant Industry, and cooperator, Bureau of Agricultural Economics, United States Department of Agriculture

INTRODUCTION

During the ginning of cotton it frequently happens that when a pull is exerted upon the tuft of fibers located at the chalazal (rounded) end of the seed, the seed coat ruptures and a fragment of the seed coat, with its attached fibers, is carried with the lint (pl. 1, *B*).

Foreign matter in lint constitutes waste most of which must be removed during the manufacturing processes. The presence of a considerable quantity of foreign matter lowers the spinning quality or value of the lint, since it adds to the difficulty and cost of manufacturing the raw cotton into yarn and may seriously affect the quality of the finished yarn and cloth.

The chalazal fragments (pl. 1, *B*), together with other seed-coat fragments of various sizes that have arisen from the cutting of seeds by the teeth of the gin saw and from motes (partly developed seeds) that have been crushed during ginning, constitute a common kind of foreign matter. These particles are particularly obnoxious, for their complete removal during manufacture is difficult. The smaller particles cling tenaciously to the lint. The larger particles, which because of their size might otherwise be more easily removed, become partly broken up during carding and combing, thus adding to the number of small particles already present. Small particles that escape the carding and combing action may become incorporated in the yarn, producing imperfections in the form of knotty bulges, dark specks, or neplike structures (pl. 1, *A*). These particles are still apparent in the woven cloth, and in dyeing cause further difficulty (*9*) [3] as their fibers may present the same problem as do neps, sometimes dyeing darker and sometimes lighter than the surrounding background. Thus, any factor that influences the extent of chipping from the chalazal end of the seed affects the spinning quality of the cotton.

It has been shown that the moisture content of the seed cotton at the time of ginning has some influence upon the number of chalazal fragments occurring in the lint and that varieties differ in the extent to which their seeds are likely to chip.[4]

[1] Received for publication September 19, 1938. This study is part of a program of work on cotton utility and standards research under the leadership of Robert W. Webb, principal cotton technologist, Bureau of Agricultural Economics.

[2] Appreciation is expressed to G. M. Armstrong, of the South Carolina Agricultural Experiment Station, through whose co-operation the plant material upon which this paper is based was grown and periodically tagged.

[3] Italic numbers in parentheses refer to Literature Cited, p. 872.

[4] SMITH, W. S., and PEARSON, NORMA L. FRAGMENTS FROM THE CHALAZAL END OF THE COTTON SEED, THEIR FORMATION, AND SOME OF THE FACTORS AFFECTING THE EXTENT TO WHICH THEY ARE PRESENT IN GINNED LINT. Unpublished manuscript.

The present paper describes the structure of the chalazal end of the cotton seed and suggests one explanation as to why seeds chip at their chalazal ends.

MATERIALS AND METHODS

The materials used in this study represented stages in the development of ovules and seeds of three cottons, Super Seven and Acala (*Gossypium hirsutum* L.) and Pima (*G. barbadense* L.). Although these cottons were not originally grown and preserved for this particular problem, it was thought that they represented a sufficient range in variety and species to form a basis for conclusions regarding the characteristics of the chalazal tissues of the cotton seed.

The plants were grown at Clemson College, South Carolina. Unopened flowers were tagged the day before flowering and were labeled as of the day of flowering. Tagging was begun the last of July and continued at 2- or 3-day intervals until the middle of September. When the first tagged bolls were mature, material from different tagging dates, representing different stages in the development of ovules and seeds, was gathered, and portions were preserved in one or more of several different fixatives—Flemming medium, medium chromoacetic, formal-acetic-alcohol, and formalin alcohol. All of the young material (buds to 10-day-old seeds) and portions of each of the older stages were embedded in paraffin in the usual manner. The remaining material was left in 70-percent alcohol. Young stages and certain of the older ones were stained with Flemming's triple stain. Other sections of the older material were mounted unstained or were stained with eosin, gentian violet, safranine, and orange G, alone or in various combinations. Mature seeds also were gathered and dried.

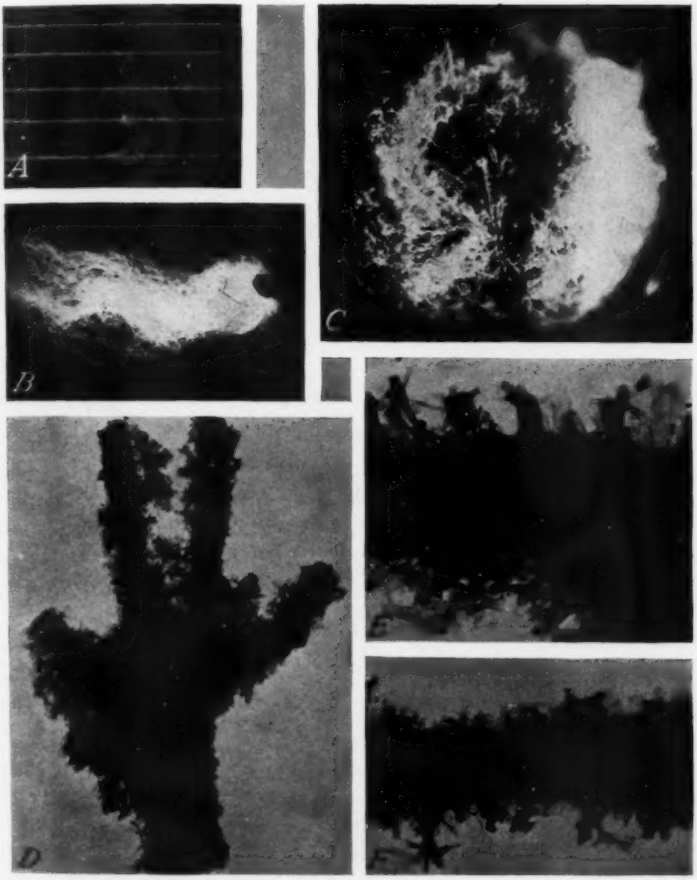
Considerable difficulty was encountered in obtaining unbroken sections of the chalazal end of seeds older than about 30 days. The tissue at the chalazal end of the seed is very brittle in its natural state and when hardened, as is necessary in the process of fixing and embedding, this brittleness is undoubtedly accentuated.

OBSERVATIONS AND CONCLUSIONS

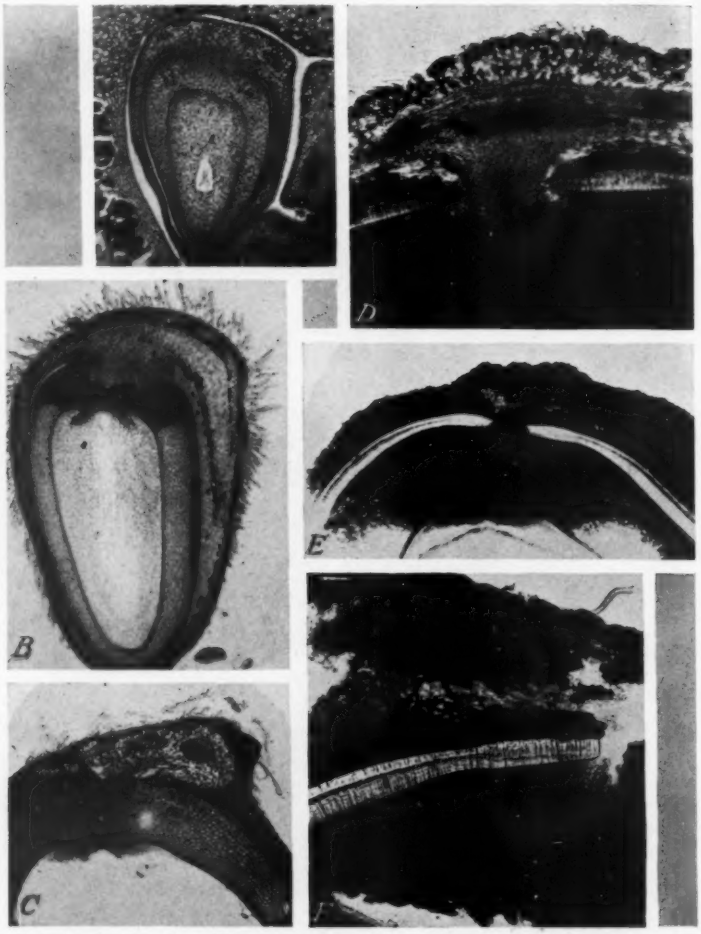
The general structure and development of the cotton seed and cotton seed coat, as revealed by these studies, are essentially the same for Acala, Super Seven, and Pima. There is some evidence of slight differences in rate of development of certain tissues, yet seeds of equal ages from one cotton also exhibit similar slight differences.

As the cotton ovule, its development into a mature seed, and the structure of the mature seed coat have been described and discussed by other investigators (*1*, *2*, *5*, *6*, *7*, *10*, *11*, *13*), only a brief description, sufficient for orienting the following discussion, will be presented.

The ovule (figs. 1, *A;* pl. 2, *A* and *B*) is anatropous. Thus, the chalazal end of the ovule is the end farthest away from the point of attachment of the ovule to the placenta, and the micropylar end is nearest. Two integuments arise from the chalaza and surround the nucellus. At the micropylar end, the outer integument overlaps the inner one to such an extent that the micropyle is not a straight passageway between the lips of the two integuments (fig. 1, *A;* pl. 2, *A*). A ridge runs along one side of the ovule. This ridge is the raphe and is formed by the fusion of the funiculus with the outer integument.



A, Irregularities in yarn due to seed-coat fragments. × 2. *B*, Chalazal fragment in ginned lint. × 2½. *C*, Chalazal region of a seed, showing the area from which a portion of the seed coat has been broken away. Note the vascular strands. × 9½. *D*, Vascular strands and surrounding spongy tissue that have been dissected away from a seed similar to *C*. × 36. *E*, Spongy tissue surrounding dissected chalazal vascular strands of 37-day-old Acala seed. × 127. *F*, Same as *E* only from a mature seed of Super Seven. × 127.



A, Longitudinal section through young ovule sectioned at right angles to raphe, Super Seven. × 42. *B*, Longitudinal section through a 2-day-old seed, Super Seven, × 33. *C*, Chalazal portion of a longitudinal section through a 12-day-old seed (fig. 2, *A*), Super Seven. × 21. *D*, Chalazal region of a section through a 22-day-old seed, Acala (fig. 2, *B*). × 42. *E*, Chalazal region of 26-day-old seed, Acala (fig. 2, *C*). × 21. *F*, Portion of chalazal region of 51-day-old seed, Pima. × 60.

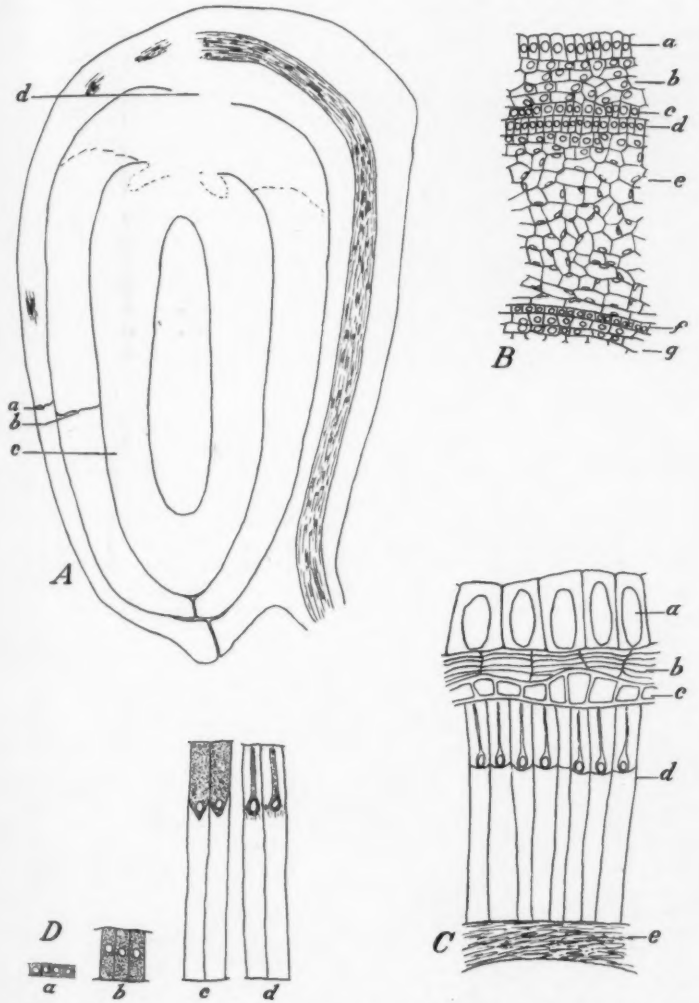


FIGURE 1.—*A*, Diagrammatic longitudinal section through a 1- to 2-day-old seed: *a*, Outer integument; *b*, inner integument; *c*, nucellus; *d*, chalaza. *B*, Section through the integument of ovule on the day of flowering: *a*, *b*, and *c*, Outer integument; *a*, outer epidermis; *b*, parenchymatous tissue; *c*, inner epidermis; *d*, *e*, and *f*, inner integument; *d*, outer epidermis; *e*, parenchymatous tissue; *f*, inner epidermis; *g*, nucellar tissue, × 207. *C*, Section through mature seed coat (semidiagrammatic); *a*, epidermis; *b*, outer pigment layer; *c*, colorless layer; *d*, palisade layer; *e*, inner pigment layer, × 104. *D*, Four stages in development of palisade layer; *a*, 1-day-old seed; *b*, 14-day-old seed; *c*, 28-day-old seed; *d*, 48-day-old seed.

The seed coat of the mature cotton seed develops from the two integuments. Before differentiation takes place, each integument consists of an outer and inner epidermis, and between them there is parenchymatous tissue (fig. 1, *B;* pl. 2, *A;* pl. 3, *C*). The thickness of the parenchymatous tissue varies somewhat at different positions on the seed (pl. 2, *B*). In the outer integument it is traversed by fibrovascular strands.

On the day of flowering or during the next few days (*3, 8*) the outer wall of certain cells of the outer epidermis of the outer integument begins to swell. These swellings elongate and eventually develop into the fibers that cover the mature seed. The epidermal cells that do not develop fibers enlarge considerably and in the mature seed coat are rather irregularly shaped (in surface view) with very thick walls and dark-colored contents.

Beneath the epidermis of the mature seed coat are, successively, the outer pigment layer, the colorless or crystal layer, the palisade layer, and the inner pigment layer (fig. 1, *C;* pl. 3, *D*).

The outer pigment layer, socalled because of the dark contents of its cells, consists of the compressed, somewhat disorganized parenchymatous tissue of the outer integument (fig. 1, *C, b*).

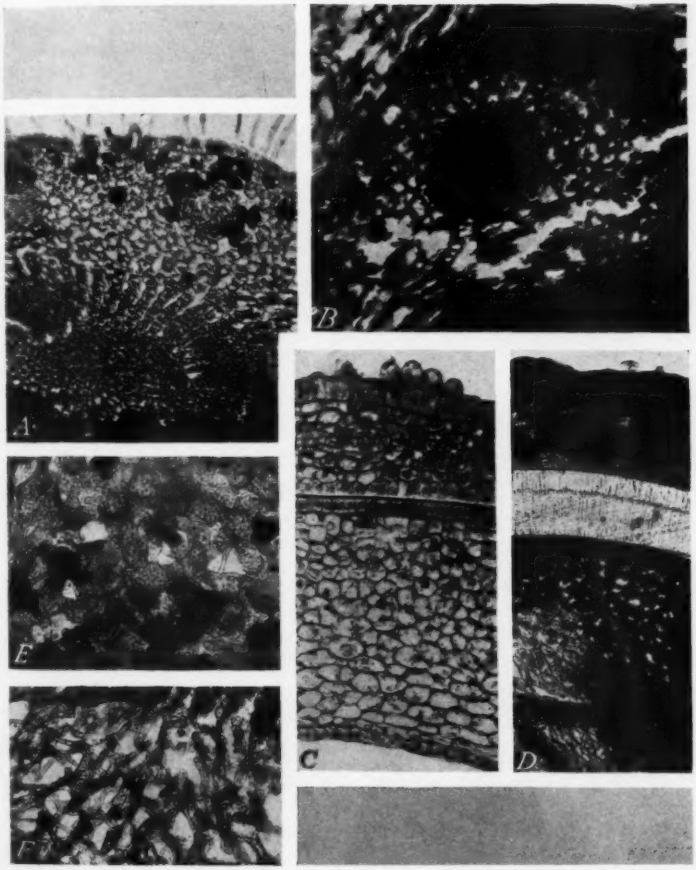
The colorless layer is derived from the inner epidermis of the outer integument (fig. 1, *C, c;* pl. 3, *D*). It is usually one, although occasionally two, cells in thickness. The cells have thick walls, and frequently crystals of calcium oxalate occur in the cell cavities (*6*).

The palisade layer gives strength and hardness to the seed coat. It develops from the outer epidermis of the inner integument (fig. 1, *B, d;* pl. 3, *C*). The cells of this epidermal layer begin to elongate in a radial direction at about the twelfth day after flowering (*1, 13*) (fig. 1, *D*), and eventually a layer is formed that in mature seeds varies in thickness from 146μ to 243μ, depending upon the species or variety (*4, 7*) and perhaps upon environmental conditions as well. About the twenty-first day after flowering, the walls of the elongating cells begin to thicken at their inner ends (*1, 13*). This thickening continues outwardly until a solid deposition is formed in approximately the inner two-thirds of each cell (fig. 1, *D, c;* pl. 3, *D*). In the external third, secondary thickening is laid down in the form of rounded ridges (*11*), leaving a small lumen that is somewhat larger at its inner end where the nucleus lies (fig. 1, *D, d*).[5] In mature seeds, the palisade layer is strongly lignified, the exact distribution of the lignin differing somewhat with different species (*7, 11*).

The inner pigment layer is derived from the parenchymatous tissue of the inner integument. It resembles the outer pigment layer, in that its cells have dark contents and are somewhat crushed and disorganized. For the most part it is thin, but at the chalazal end it is much thickened and forms a little cushion (pl. 2, *E*) (*2, 6, 11*).

The inner epidermis of the inner integument forms a layer known as the "fringe" layer. This layer does not belong to the seed coat proper, but together with the perisperm and the remains of the endosperm, forms a sheath that surrounds the embryo. The cells of the mature fringe layer are somewhat elongated, and there are projections on their walls that extend into the lumen (*6, 11*).

[5] It has been reported that the ratio between the lengths of the inner and outer portion of the palisade cells of mature seeds may vary with species and varieties (*4*).



A, Chalazal region of a section through a 7-day-old seed, showing the more or less distinct strands of linearly arranged cells associated with the chalazal plug (the section is not a true longitudinal one), Super Seven. × 125. *B*, Vascular strand in the chalazal region, surrounded by spongy tissue (pl. 2, *C*), Super Seven. × 94. *C*, Section through testa of a 2-day-old seed (fig. 1, *B*), Pima. × 219. *D*, Section through seed coat of a 41-day-old seed (fig. 1, *C*), Pima. × 63. *E*, Chalazal spongy tissue of an 8-day-old seed, Pima. × 219. *F*, Chalazal spongy tissue from 22-day-old seed, Pima. × 94.

Earlier investigators considered the fringe tissue to have arisen from the epidermis of the nucellus (*2, 6, 13*), but recent work of Reeves (*10*) has shown that this layer is formed from the inner epidermis of the inner integument and cannot be considered as belonging to the perisperm. The author's investigations confirm the findings of Reeves.

The description given above applies to the greater portion of the mature seed coat. The chalazal portion, however, is somewhat different in structure. In a view of the chalazal region of a longitudinal section through a mature cotton seed, the characteristic that stands

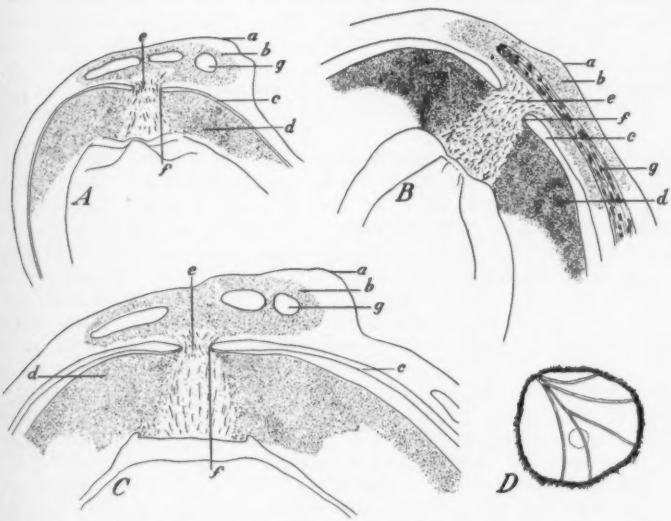


FIGURE 2.—*A*, *B*, and *C*, Chalazal portions of longitudinal sections through seeds (semidiagrammatic), × 21: *A*, 12-day-old seed. *B*, 22-day-old seed. *C*, 32-day-old seed. *D*, Vascular strands in the chalazal portion of the seed, seen after dissecting away the overlying tissues. *a*, Epidermis; *b*, outer chalazal spongy tissue (dotted); *c*, palisade layer; *d*, inner spongy tissue (dotted) of chalazal cushion; *e*, chalazal plug; *f*, strands of linearly arranged cells; *g*, vascular tissue.

out most strikingly is that the palisade layer is not continuous. It ends abruptly, leaving a very small round opening (fig. 2, *B* and *C*; pl. 2, *E*). The structure of the portion of the palisade layer surrounding this opening is peculiar. The individual cells in certain definite portions differ in length from the cells in certain other portions, resulting in variations in the thickness of the palisade layer. As the layer curves up over the broadened part of the seed, it gradually becomes thinner (the individual cells being progressively shorter) and then nearer the opening thickens somewhat (the individual cells being progressively longer) (fig. 2, *C* and *B*; pl. 2, *E* and *F*). This thickened part extends for a short distance before the layer becomes slightly thinner and then ends abruptly (pl. 2, *F*).

In addition to the variations in length of the cells composing the chalazal portion of the palisade layer, there is also a variation in the amount of solid deposition in the inner portion of each cell. Passing

toward the opening from the region where the palisade layer first grows thinner, the solid portion in each cell is seen to grow progressively shorter until, in the thickened portion near the opening, it occupies only about the inner one-fourth or one-fifth of each cell (fig. 2, *C*; pl. 2, *F*).

The opening in the palisade layer is nearly filled with a core of compact undifferentiated cells (pl. 2, *C*, *D*). This core has been called, for the sake of convenience, the chalazal plug.

The explanation for the opening in the palisade layer is to be found in the structure of the ovule. The palisade layer is derived from the outer epidermis of the inner integument. This epidermis ends at the point where the integument arises from the chalaza. Consequently, the palisade layer ends at the chalaza. The chalazal plug thus represents tissue of the chalaza proper.

Examination of the seed-coat tissues reveals that the fringe tissue and the colorless tissue, both of which are derived from epidermal tissues, end at the chalaza.

Between the abrupt ending of the palisade layer and the solid tissue of the plug is a ring of loosely arranged cells, one cell in thickness (pl. 2, *D*). This ring flares out to the inside and to the outside of the opening left by the ending of the palisade layer, forming a sort of sheath made up of more or less separate strands of linearly arranged cells. This linear arrangement of the cells can be most easily demonstrated in young material, for in mature seeds the cells crumble in sectioning. If young seeds are cut longitudinally and to one side of the median of the plug, the separate rows of cells usually can be seen (pl. 3, *A*).

The sheath composed of linearly arranged cells, together with the tissue of the plug, merge externally and internally with tissues which, though corresponding in position to the outer and inner pigment layers, differ in structure from these layers at other parts of the seed coat.

It has been said previously that the inner pigment layer is very much thickened at the region of the chalaza and forms a little cushion (*2*, *6*, *11*). The tissue of this cushion is spongy (pl. 3, *D*) except in the part extending directly between the chalazal plug and the nucellus. The individual cells of the cushion have been described as star-shaped (*2*, *6*). Even in very young seeds, the chalazal tissue that will form this cushion is characterized by the dark contents of its cells (pl. 2, *B*) and by the presence of small intercellular spaces.

The chalazal cushion connects by means of the chalazal plug and its surrounding rows of cells with the tissue external to the palisade layer. This external tissue is even more spongy than the tissue of the cushion (pl. 2, *C*) and will hereafter be designated as the "spongy" tissue.

Very young seeds give evidence of the eventual spongy character of this tissue. Seeds 1, 2, or 3 days old show the tissue in this region to be less compact than the adjacent parenchymatous tissue of the outer integument. The cells are rounded, and small intercellular spaces occur. As the seeds develop, these intercellular spaces increase in size in such a way as to make a tissue the cells of which, though frequently rather elongated, are somewhat star-shaped, each cell possessing several arms that connect with similar arms from adjacent cells (pls. 3, *E* and *F*; 1, *E*).

As the development of the seed progresses, the starlike form of the cells of the spongy tissue becomes more and more pronounced. The

cell walls remain thin; and when the mature seed dries, the cells shrivel to a certain extent but still retain to a greater or less degree their star-shaped appearance (pl. 1, *F*). A similar type of development is also taking place in the tissue that will form the chalazal cushion in the mature seed; but the intercellular spaces here do not become so large, nor the projecting arms so delicate, as in the outer tissue.

In the immediate vicinity of the chalazal plug, most of the tissue (exclusive of the vascular strands) between the palisade layer and the epidermis is composed of spongy cells (pl. 2, *C*). The region occupied by these cells extends entirely to or within two or three cells of the epidermis and within a few cells of the palisade layer; it extends outwardly beyond the chalazal plug for varying distances, becomes thinner, and gradually merges with the more solid tissue of the outer pigment layer (pl. 2, *C* and *D*).

The chalazal epidermis is not essentially different from the epidermis of the rest of the seed coat except that stomata and fibers are more numerous (*12*) than elsewhere on the seed.

The single vascular strand that extends through the raphe branches at a point a little to one side of the chalazal plug into several strands that fan out through the chalazal spongy tissue and extend onward through the outer pigment layer (outer integument). Thus in the vicinity of the chalazal plug and for various distances around it, the vascular strands are surrounded by cells of the spongy tissue (pls. 1, *D*; 2, *C*; 3, *B*).

By comparing the position of the spongy tissue in unbroken seeds with the position of the broken area in seeds that have undergone chalazal chipping during either hand ginning or machine ginning, it is obvious that the fragments pulled away include a portion of the spongy tissue. Moreover, the chalazal fragments found in ginned lint show the ragged edges of the broken cells of the spongy tissue, indicating that it is in the spongy tissue that the main rupture occurs.

The broken areas vary in size in different seeds, but careful examination shows that they usually are very close to or include the region over the chalazal plug. Occasionally chipping occurs at the chalazal end of the raphe, and the broken area may include the point at which the raphe strand branches.

If chipping occurs when the seed coat is moist, the vascular strands frequently are left more or less intact on the seed (pl. 1, *C*), indicating that the ruptures occurred in the spongy tissue between the vascular strand and the epidermis. But with seeds that were chipped when the seed coat was fairly dry, the vascular system occasionally may be left fairly intact. Usually, however, it is either entirely torn away or only small fragments are left, the rupture in such cases occurring in the inner portion of the spongy tissue; that is, between the vascular strands and the palisade layer.

Undoubtedly, the spongy tissue constitutes a weak place in the chalazal portion of the cotton seed coat, and its existence furnishes one explanation why cotton seeds rupture at their chalazal ends during ginning.

It must not be overlooked, however, that there may be factors other than the existence of this spongy tissue that aid in bringing about a rupture of the seed coat. The epidermis itself is undoubtedly weakened by the greater number of both fibers and stomata at the chalazal end of the seed (*12*). Thus, an original rupture in the epidermis

resulting when force is applied to the attached fibers, might extend beyond the epidermis to the easily broken layer below it, pulling away a fairly large layer of the chalazal epidermis with the attached fibers and parts of the spongy tissue and vascular system.

SUMMARY

During ginning, fragments of the cotton seed coat with the attached fibers may be broken from the chalazal end of the seed, thereby adding undesirable foreign matter to the ginned lint.

Material representing different stages in the development of the seeds of three cottons—Super Seven and Acala (*Gossypium hirsutum* L.) and Pima (*G. barbadense* L.)—showed that the chalazal part of the seed coat has a very characteristic structure.

The palisade layer—since it is derived from the outer epidermis of the inner integument—ends at the point of attachment of the inner integument to the chalaza. Thus a small opening is left in the palisade layer, which is nearly filled with a fairly compact mass of undifferentiated cells, the chalazal plug.

The inner and outer pigment layers are thicker in the chalazal region than elsewhere on the seed and are spongy in nature. The outer pigment layer is much more strikingly spongy than the inner pigment layer, the intercellular spaces being larger and the connecting arms more delicate. The spongy portion of the outer pigment layer extends over the chalazal end of the seed for various distances, eventually merging with the more solid tissue comprising the rest of the layer. This outer spongy tissue is traversed by branches from the single vascular strand of the raphe.

In chalazal chipping, the main rupture occurs in the spongy tissue—either above or below the vascular strands. The broken areas vary in size in different seeds, but usually include a portion of the spongy tissue very close to or over the chalazal plug.

This outer spongy tissue constitutes a weak place in the seed coat and its existence furnishes one explanation for the chipping of cotton seeds at the chalazal end during ginning.

LITERATURE CITED

(1) Balls, W. Lawrence.
1915. The development and properties of raw cotton. 221 pp., illus. London.

(2) Bretfeld, [F.] von.
1887. Anatomie des Baumwolle und Kapoksamens. Untersuchungen zum zwecke der construction von verfälschungs, identitäts- und qualitätsdiagnosen. Jour. Landw. 35: [29]–56, illus.

(3) Farr, Wanda K.
1931. Cotton fibers. I. Origin and early stage of elongation. Contrib. Boyce Thompson Inst. 3: 441–458, illus.

(4) Haller, R.
1919. Mikroskopische Diagnostik der Baumwollarten; versuch einer diagnostizierung der einzelnen Baumwollspezies in der rohen Baumwolle, dem rohgespinst und rohgewebe. 52 pp., illus., Wittenberg.

(5) HANAUSEK, T. F.
1888. ZUR MIKROSKOPISCHEN CHARACTERISTIK DER BAUMWOLLESAMEN-PRODUCTE. Ztschr. Allg. Österr. Apoth.-Ver. 26: 569–572, 591–595, illus.
(6) ———
1903. SAMEN. ÜBERSICHT DER GEWÄCHSE DEREN SAMEN TECHNISCH BENUTZT WERDEN. *In* Wiesner, Julius, Rohstoffe des Pflanzenreiches, Versuch einer technischen Rohstofflehre des Pflanzenreiches, Aufl. 2, Bd. 2, pp. [685]–781, illus. Leipzig.
(7) KONDŌ, MANTARŌ.
1925. ÜBER DIE IN DER LANDWIRTSCHAFT JAPANS GEBRAUCHTEN SAMEN. (FÜNFTE MITTERLUNGEN). BESONDERS ÜBER MALVACESAMEN. Ber. Ōhara Inst. Landw. Forsch. 2: [559]–595, illus.
(8) LANG, A. G.
1938. THE ORIGIN OF LINT AND FUZZ HAIRS OF COTTON. Jour. Agr. Research 56: 507–521, illus.
(9) MIDGLEY, E.
1933. DEFECTS IN DYED COTTON GOODS. A CONSIDERATION OF SOME OF THE CAUSES. Textile Weekly 11: 20–21.
(10) REEVES, R. G.
1935. ORIGIN OF THE FRINGE TISSUE OF THE COTTON SEED. Bot. Gaz. 97: 179–184, illus.
(11) ——— and VALLE, C. C.
1932. ANATOMY AND MICROCHEMISTRY OF THE COTTON SEED. Bot. Gaz. 93: 259–277, illus.
(12) WILLIAMS, C. B.
1927. FIBER DISTRIBUTION ON THE SEED COAT OF COTTON. N. C. Agr. Expt. Sta. Ann. Rept. 49: 24–26, illus.
(13) WITTMACK, LUDWIG.
1928. BOTANIK UND KULTUR DER BAUMWOLLE. 352 pp., illus. Berlin.

151679—39——7

U. S. GOVERNMENT PRINTING OFFICE: 1939